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Case Report

Atresia ani associated with recto-vaginal fistula in two months old Sudanesse crossbreed lamb

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A case of atresia ani with recto-vaginal fistula was observed in a two-month old Sudanese crossbred lamb. On physical examination, the abdomen was distended, and there was bulging around the perineal region and severe straining. Upon palpation, the anal ring was felt at the anal area, voiding of feaces and urine from the vaginal opening was seen, while the fistula was felt upon vaginal palpation. Reconstructive surgery by incising the bulged, palpable region was done. The rectum was located and sutured to the perineal wall. The patency of the newly constructed anal opening was however maintained with the use of fabricated syringe barrel. Full recovery was attained within 21 days. The animal was relived of pressure strain, pain through the surgical intervention with resultant increase in body condition score.

Key words: Atresia ani, fistula, anal, palpation.

INTRODUCTION

Congenital anomalies of gastro intestinal tract occurs among different species of animals with an incidence of about 4.3% (Leipold et al., 1971).

Atresia ani is a developmental anomaly of the new born which occurs as a result of an autosomal recessive gene (Bademkiran et al., 2009). This condition is characterized by absence of anal opening and may be associated with recto-vaginal fistula, recto-cystic fistula, vagino urethral agenesis, taillessness, hypospadias (Singh et al., 1993) and diphallus (Loynachan et al., 2006). Recto-vaginal fistula or anus vaginalis is an inherited lethal abnormality in which there is an abnormal passage between rectum and vagina; also, feaces are passed through the vagina as a result of the imperforate anus (Oehme and Prier, 1974). Atresia ani associated with recto-vaginal fistula has been reported in many species. These include calves (Shakoor et al., 2012; Mahesh et al., 2014), lambs (Kamalakar et al., 2014, 2015), dogs (Rahal et al., 2007) and pigs (Monsang et al., 2014).

These anomalies are usually noticed at birth whereas in some cases, usually diagnosed at a later age. Early diagnosis of non-lethal anomalies aids in efficient

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Table 1. Haematology (complete blood count).

Parameter	Test results
PCV (%)	32
Hb (g/dl)	10.6
RBC (× 10^{12}/L)	8.3
MCV (fl)	52.1
MCHC (g/dL)	33.3
HCT	
MCH(pg)	16.8
Platelets(×10^3/L)	
WBC (10^9/L)	6.9
Neutrophil-Band	
Neutrophil-Mature	31
Lymphocytes	63
Monocytes	2
Eosinophil	4
Basophil	0

All hematological values are within the normal range.

management of the condition.

There are four major types of atresia ani which consists of type I-IV. In type I, a mucosal barrier obstructs the lumen of the intestine. Animals with type II have an intestine with two segments without any communication usually with a fibrous cord joining them together. In type III, two segments of intestine separated completely which may be coiled at the distant end in some animals. Type IV atresia involves multiple site of atresia (Bademkiran et al., 2009; Rahal et al., 2007). Congenital rectovaginal fistula usually associated with type II atresia ani in which the rectum ends as a blind pouch immediately cranial to the imperforated anus (Rahal et al., 2007).

CASE HISTORY AND CLINICAL EXAMINATION

A 2-month old female Sudanesse crossbred lamb was presented to the Veterinary Teaching Hospital, University of Maiduguri, Nigeria with the complaint of inability to void feaces normally. An examination of perineal region, revealed absence of anal opening, tenesmus, bulging at the anal region and communication between rectal floor and vaginal roof, through which the feaces was voiding out. Based on the prevailing clinical signs, the condition was however diagnosed as congenital atresia ani associated with recto-vaginal fistula.

Treatment plan

Complete blood count was conducted so as to ascertain whether the animal is anemic or not; ascultation of the heart and lungs was done to know the status of the cardiopulmonary system, whether or not the animal can withstand both aesthesia and surgery. Unfortunately, ultrasound scan was not done to know the type of atresia ani we were dealing with and lastly, reconstructive surgery was performed to correct the condition.

Vital parameters

Respiratory rate: 65 breaths/min (60-90)
Heart rate 120 beats/min: (70 - 100)
Temperature: 38.5 (38 - 40) (Table 1).

Surgical treatment

The rectum and vagina were evacuated of feaces, the perineum was shaved and prepared for aseptic surgery (Figure 1). Fluid therapy using 5% normal saline was instituted. Anesthesia was effected with xylazine at 0.084 mg i.m. and local infiltration of the perineum using 20% lignocaine hydrochloride. The animal was placed on lateral recumbency and a cruciate skin incision was made on the skin at the bulging area. The rectum was opened and the contents were evacuated. The fistulous orifice, which was about 3 cm in diameter and 2” away from anus, was reached through anal route and was closed using simple interrupted suture pattern with chromic catgut size 0/2 (Figure 2). The area was irrigated with normal saline and rectal mucosa was sutured to the skin

Figure 1. Black arrow shows the fistula opening between rectum and vagina.

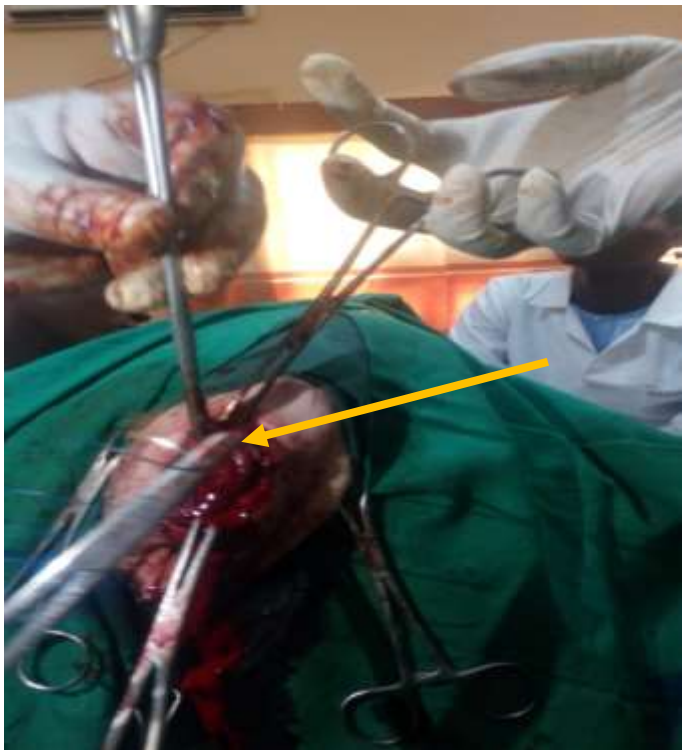

Figure 2. Arrow shows suturing of fistula No. 2 (3.5 metric) using simple interrupted pattern.

Figure 3. Arrow shows syringe barrel.

in simple interrupted pattern using black braided silk. A sterile 20-mL syringe barrel (Figure 3) was cut at non winged end and two holes were made at the centre of each wing. The non-winged end was lubricated with liquid paraffin and inserted into rectum in other to hold the rectum tightly to the skin, to avoid closure through healing. The wings of barrel were secured to the perineal skin by passing nylon suture material from skin through hole in that side wing and tied to outside using simple interrupted sutures.

Outcome

The animal fully recovered 90 min after the surgery and stood on its entire limbs (Figure 4). After another 90 min, a rising appetite was noticed, hence small quantity of feed (wheat bran) was provided. After 24 h, normal defecation even though softer than normal was noticed, which may be due to the wheat bran.

Post-operative care

Post operatively, 5% dextrose for rehydration, procine penicillin with streptomycin was given at 9 mg/kg × 3/7. Diclofenac sodium was given at 3 mg/kg × 3/7 as an analgesic. The animal was fed wheat bran continuously for 7 days at the Veterinary Hospital so that it could pass out soft feaces and for monitoring, should there be any complication.

Figure 4. Recovery of the lamb after surgery.

DISCUSSION

Atresia usually arises during the embryonic period which results from autosomal recessive gene (Loynachan et al., 2006). Though environmental teratogens, plant toxins and some viruses (Loynachan et al., 2006) are recognised complicating factors in calves. In the present case, the reason could not be ascertained and unspecific as reported by Johnson et al. (1980). The increased faecal pressure may have caused an abnormal opening between rectal wall and vagina forming recto-vaginal fistula and thus causing defecation via vulva (Norrish and Rennie, 1968). Atresia ani is frequently associated with recto-vaginal fistula between dorsal wall of vagina and ventral wall of terminal rectum. A clinical sign is mainly the absence of anal opening; however, while Amith et al. (2017) reported tenesmus, abdominal discomfort in a 5-day old lamb, Prasad et al. (2016) did not report such clinical signs in a 3-day old lamb. Tenesmus, abdominal distension, abdominal discomfort was equally not observed in this present study, which means there are variations in clinical signs regardless of age. Furthermore, radiographs are considered important to determine the position of the fistula and to differentiate the four types of congenital atresia ani (Rahal et al., 2007). However, in our present case, the defects were rectified individually as reported by Rahal et al. (2007).

Conclusion

Surgical intervention (anal reconstruction) is the only possible solution to cope with these congenital anomalies in animals and to make affected animals economically profitable for the keepers. However, it is worthy of note that female animals, especially the Sudaneese breed with these conditions may live up to 2 months or more and still have a good body condition score until corrected.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Amith NG, Sandesh KV, Nagaraja BN (2017). Surgical repair of congenital rectovaginal fistula and atresia ani in a lamb. International Journal of Science and Research pp. 12-14. Available at: https://www.ijsr.net/archive/v6i9/ART20176515.pdf

Bademkiran S, Icen H, Kurt D (2009). Congenital recto vaginal fistula with atresia ani in a heifer: a case report. YYU Veteriner Fakultesi Dergisi 20(1):61-64.

Johnson EH, Nyack B, Marsh A (1980). Surgical repair of atresia ani and rectovaginal fistula in a goat. Veterinary medicine, Small Animal Clinician 75(12):1833.

Kamalakar G, Devaratnam J, Brahmaji R, Jyothsna B (2015). Congenital atresia ani associated with recto-vaginal fistula in Ongole calf. Journal of Livestock Science 6:80-84.

Kamalakar G, Sumiran N, Mahesh R, Prasad VD (2014). Repair of acquired recto- vaginal fistula associated with atresia ani in a lamb. Available at: http://www.cibtech.org/J-FOOD-AGRI-VETERINARY-SCIENCES/PUBLICATIONS/2014/Vol_4_No_2/JFAV-010-030-KAMALAKAR-REPAIR-LAMB%20f.pdf

Leipold HW, Dennis SM, Huston K (1971). Congenital defects of cattle: Nature, cause, and effect. Advances in Veterinary Science and Comparative Medicine 16:103-150.

Loynachan AT, Jackson CB, Harrison LR (2006). Complete diphallia, imperforate ani (type 2 atresia ani), and an accessory scrotum in a 5-day-old calf. Journal of veterinary diagnostic investigation 18(4):408-412.

Mahesh R, kamalakar G, Devi Prasad V (2014). Surgical management of atresia ani in a calf: A case report. International Journal of Veterinary Science and Medicine 2(2):51-53.

Monsang SW, Madhu DN, Sarode IP, Kumar R, Amarpal, Pawde AM, Kinjavdekar P, Aithal HP (2014). Surgical repair of a rare case of congenital recto-vaginal fistula and atresia ani in a crossbred piglet. Available at: http://www.journalijiar.com/uploads/2014-09-01_170651_4.pdf

Norrish JG, Rennie JC (1968). Obervations on the Inheritance of atresia ani in swine. Journal of Heredity 59(3):186-187.

Oehme FW, Prier JE (1974). Text book of large animal surgery: Williams and Wilkins, Baltimore London pp. 425-509.

Prasad VD, Mahesh R, Kamalakar G, Devarathnam J (2016). Congenital recto-vaginal fistula with atresia ani in a lamb: A case report. Available at: http://www.ijset.net/journal/1409.pdf

Rahal SC, Vicente CS, Mortari AC, Mamprim MJ, Caporalli EH (2007). Rectovaginal fistula with anal atresia in 5 dogs. The Canadian Veterinary Journal 48(8):827.

Shakoor A, Muhammad SA, Younus M, Kashif M (2012). Surgical repair of congenital recto-vaginal fistula with atresia ani in a cow calf. Pakistan Veterinary Journal 32(2):298-300.

Singh J, Singh AP, Patil DB (1993). From Digestive system: In Ruminant Surgery 1st edition, Edited by Tyagi RPS and Singh J, CBS Publishers, New Delhi P 222.

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Full Length Research Paper

Bovine mastitis: Prevalence, Isolation and identification of major bacterial pathogens in selected areas of Bench Maji Zone, Southwest Ethiopia

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A cross-sectional study was conducted to determine the prevalence of mastitis in bovines and to, isolate and identify the major bacterial pathogens of lactating cows in six selected woredas of Bench Maji Zone of Southwestern Ethiopia from June 2017-October 2017. Three hundred eighty four lactating cows were examined for mastitis by combination of udder physical examination, California Mastitis Test and subsequent bacteriological isolation. During the study period, 116 (30.21%) cows had mastitis, of which 35 (30.17%) and 81(69.83%) showed clinical and subclinical mastitis, respectively. The prevalence rates of mastitis in cross breed and local breed cattle were found to be 71.43 and 28.65%, respectively. Based on parity, higher prevalence (45.45%) was recorded in cows which had greater than 5 parturitions and the lower prevalence (13.04%) was recorded in cows with 1-2 parturitions. Based on lactation stage, the prevalence was (45%) in late stage, followed by middle stage (36.60%) and early stage of lactation (13.85%). The prevalence rates of mastitis based on different age groups of lactating cows were found to be 48.78 , 30.54 and 18.52% in cows of greater than 8 years old, 4-8 years old and in cows less than 4 years old, respectively. Except parity, the other associated risk factors (breed, lactation stage and age group) had significant association (P<0.05) with the prevalence of mastitis in the study animals. Upon subsequent bacterial culturing, the quarter milk samples yielded three types of bacteria. *Staphylococcus aureus* (59.26%), *Streptococcus agalactiae* (38.27%) and *Escherchia coli* (2.47%) were the major isolates. In conclusion, the overall prevalence of mastitis in lactating cow of the study area was high and this suggests the need of improved hygienic practices and applies different methods for prevention and strategic control of the disease.

Key words: Bacteria, cows, Ethiopia, mastitis, prevalence.

INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country and still promising to rally round the economic

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development of the country. Among livestock, cattle play a significant socio-economic role in the livelihoods of the Ethiopian people. Livestock products (meat, milk, cheese and butter) and by-products supply animal protein that contributes to the improvement of the nutritional status of the people (CSA, 2015). Conversely, low annual per capita consumption of milk in Ethiopia (19 kg) revealed that current milk production in Ethiopia is insufficient to fulfill the requirements due to a multitude of factors (FAO, 2017).

Mastitis can reduces milk yield, increase culling rate, incur treatment cost and occasionally result in death from severe infection. Moreover, mastitis had been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield and to cause culling of animals at an unacceptable age (Radostits et al., 2007).To increase milk production cross breeding of indigenous zebu cattle with exotic breeds particularly *Holstein Friesian* is widely practiced. This resulted in a larger portion of the dairy cattle population especially in urban areas to be with a high level of exotic blood. However, this market oriented dairy production in many African countries, is subjected to diseases of intensification including mastitis and reproductive disorders (Lemma et al., 2001).

Oviedo-Boyso et al. (2007) and Suriyasathaporn et al. (2000) revealed that mastitis is a multifactorial disease. As such, its incidence depends on exposure to pathogens, effectiveness of udder defense mechanisms and presence of environmental risk factors, as well as interactions between these factors. Seegers et al. (2003) indicated that mastitis has been described as the most common and costly disease in dairy production causing over 38% economic losses due to health problems. Many infectious agents have been identified as cause of mastitis in cattle. The most common organisms being *Streptococcus agalactiae* and *Staphylococcus aureus* whereas, environmental mastitis is associated with Coliforms and environmental Streptococci that are frequently found in the cow's environment (Radostits et al., 2000; Quinn et al., 2002; Endale et al., 2016; Jafer et al., 2016; Belay and Tadele, 2017).

It is a serious problem in the dairy industry of Ethiopia (Mekonnen et al., 2005). Bovine mastitis is among the major health problem hindering dairy productivity in Ethiopia, which requires the development of methodologies of control program under the prevailing husbandry system (Fufa et al., 2013). However, the information about prevalence of the disease is inadequate. Such information is important when designing appropriate strategies that would help to reduce its prevalence and effects. From the economic point of view, mastitis especially the-subclinical form causes extensive economic losses that include reduction of milk yield, changes in the milk composition and reduction in milk as well as shortens the productive life span of the affected animals (Radostits et al., 2007).

Mastitis is one of the most important destructive infectious diseases of dairy cattle industry and it is considered of quite vital importance to the public health as it is associated with many zoonotic diseases in which milk acts as a vehicle for the infectious agents. Mastitis not only brings huge economic losses of dairy cow production, but it also cause public health and food safety. The safety of milk with respect to food born disease is a great concern around world this is especially true in developing countries like Ethiopia where the production of milk often take place under unsanitary conditions and consumption of raw milk which is typically produced in small dairy farm under unsatisfactory hygienic conditions is a common practice (FHR, 2006; Teshome and Tesfaye, 2016). Most of the studies in Ethiopia were carried out in Addis Ababa and its surrounding, which may not representative of other regions of the country (Almaw et al., 2009). In Bench Maji Zone, mastitis is commonly observed in dairy cattle. However, scientific data and literature is not available on the current status of mastitis in the targeted area. Therefore, the objectives of this study were to determine the prevalence of mastitis and to isolate and identify the most common bacteria associated with the subclinical and clinical mastitis of cows in study area.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Bench Maji zone (BMZ) of Southwestern part of Ethiopia. The zone is found at a distance of about 561 km from Addis Ababa and 830 km from the regional capital Hawassa. Agro-ecologically, BMZ consists of 52% lowland (<1500 m above sea level (masl), 43% mid altitude (1500-2300 masl) and 5% highland (>2300 masl). The zone s found at 34°45'-36°10' east and 5°40'-7°40' north. The annual average temperature ranges from 15.1 to 27.5°C, while the annual rainfall ranges from 400 to 2,000 mm. The total cattle, sheep and goats population in the zone is about 334,502, 181, 203 and 93,952, respectively (CSA, 2016/2017).The study was conducted in six *woredas* of the zone namely Sheko, Guraferda, Debub Bench, Shey Bench, Semen Bench, Menitgoldia and Maji.

Study animals

The study was conducted on lactating local (indigenous zebu, Sheko) and cross breed cows that were managed under extensive, semi intensive and intensive farming system.

Study design

A cross-sectional study was carried out in June 2017 - October 2017 to investigate the prevalence of mastitis and to isolate and identify the most common bacteria associated with the subclinical and clinical mastitis of cows in study area.

Sampling method and sample size

Out of 10 *woredas* of the BMZ six *woredas* were selected purposively based on accessibility for transportation of milk samples

and population of cattle. From each *woreda*, two *kebeles* were selected purposively and the household with at least one lactating cow was involved in the study. From each selected *kebele,* 32 lactating cows were selected by using simple random sampling method for CMT and bacteriological examinations. The sample size for the study is calculated based on the formula developed by Thrustfield (2007) for random sampling method. A 5% absolute precision and 95% confidence interval was used for determining sample size. Since there is no previous study on the prevalence of mastitis in the study areas, an expected prevalence of 50% was used to determine the maximum sample size.

$$n=\frac{(Z\frac{\alpha}{2})^2\ p(1-p)}{Sd^2} \qquad n=\frac{(1.96)^2\ 0.5(1-0.5)}{(0.05)^2}\ = 384.16$$

Where, P= is the expected prevalence, Sd = is standard deviation (desired absolute precision).
n = the total sample size

Accordingly, the calculated sample sizes was 384 samples.

Clinical examination

Physical examination for evidence of clinical mastitis was conducted in all lactating cows that were sampled in the study area. The udders of the selected cows were examined visually and by palpation for any presence of clinical mastitis. During examination, attention was paid to cardinal signs of inflammation, blindness, injuries, milk clots, symmetry, size, consistency of udder quarters and swelling (Radostits et al., 2007). A cow was considered to have clinical mastitis if it fulfilled at least two of the clinical findings, (1) pain reaction upon palpation, (2) changes in colour and consistency of milk (blood tinged milk, watery secretions, clots, pus) and (3) change in consistency of the udder (Lakew et al., 2009). Cows that did not have clinical mastitis were tested further for sub-clinical mastitis based laboratory investigation.

Milk sample collection and Laboratory investigation

According to Quinn et al. (2002) procedures of mastitis testing, the lactating cows' milk samples were directly collected using universal sample collection bottles. The first 3-4 streams of milk were discarded. The collecting bottle was held as near horizontal as possible and by turning the teat to a near horizontal position and approximately 10 ml of milk were collected into the container. After collection, the sample was labeled and placed in ice box and transported to the Mizan Regional Veterinary Diagnostic Laboratory. The analysis was performed within two to three hours after sampling.

California mastitis test (CMT)

The CMT was conducted to diagnose the presence of subclinical mastitis (Quinn et al., 1999). Collected milk samples were poured in to four shallow cups in the CMT paddle and equal amount of CMT reagent was added to each cup and gentle circular motion was applied to the mixture on the horizontal plane. Based on the thickness of the gel formed by CMT reagent and milk mixture, test results were scored as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Milk samples with test result of CMT 1 to 3, was classified as evidence of subclinical mastitis (Quinn et al., 1999; Radostits et al., 2007).

Bacterial isolation and identification

Bacteriological study was performed on milk samples from the sepositive CMT reactive and mastitis milk for culture. Identification of mastitis pathogens was carried out following microbiological procedures for diagnosis of bovine udder infection described in Quinn et al. (1999). One standard loop (0.01 ml) of milk was streaked on 7% blood agar. The inoculated plate was incubated aerobically at 37°C. The plates were checked for growth after 24, 48 and up to 72 h to rule out slow growing bacteria species. A milk sample was considered positive for mastitis pathogens if at least single colonies of a potential pathogen were detected and the positives were identified by biochemical tests. For primary identification, size, shape, color, hemolytic characteristics, Grams reaction and catalase production was used. For confirmation, biochemical tests were used after sub culturing isolated distinct colony on selective media. MacConkey agar (Oxoid) and Edward's agar (Oxoid) were used to detect the most aerobic pathogens, enteric bacteria and *Streptococci,* respectively. Primary identification of *Staphylococci* was based on colony morphology, catalase test, Gram-staining morphology and differentiated from micrococci on the basis of the oxidative fermentative (OF) test carried out on semi-solid OF medium(Difco, Becton, Dickinson and Company, Franklin Lakes, NJ,USA). The *Staphylococci* were also tested for production of coagulase enzyme by the tube method as described by Quinn et al. (1994).

Isolates that produced Gram-positive cocci in clusters, and were catalase positive, glucose-fermentative, resistant to bacitracin and did not produce coagulase were identified as coagulase-negative staphylococci (CNS). *S. aureus* isolates were differentiated from other coagulase-positive staphylococci on the basis of mannitol fermentation on mannitol salt agar (Oxoid). The enteric bacteria were identified using colony morphology, oxidase test, lactose fermentation on MacConkey agar (Oxoid), indole production test, citrate utilization Quinn et al. (1999). Interpretation was made according to NMC (1990). The culture was considered negative if no growth occurs after 72 h of incubation and plates showing mixed and confluent growths, with no evidence of single discernible colonies, were not investigated further.

Data analysis

Data collected from the laboratory test and the questionnaire survey was recorded and coded in Microsoft excel spread sheets 2010 and analyzed using statistical data analysis of SAS version 9.10. The prevalence of mastitis was calculated as the number in study population testing positive divided by the total study units tested. The Chi-square (χ^2) test was applied to determine existence of any association between the laboratory test positivity and the associated risk factors (such as breed type, parity, lactation stage and age of milking cows). For all analysis, a P-value of less than 0.05 was taken as significant.

RESULTS AND DISCUSSION

Prevalence of mastitis

The overall prevalence of mastitis of cow level in the study areas were tested by using CMT and clinical inspection of the udder (Table 1). From the total 384 lactating cows examined during the study period, 116 (30.21%) cows had mastitis, of which 35 (30.17%) and 81(69.83%) showed clinical and subclinical mastitis, respectively. In the current study, the clinical and

Table 1. Clinical and subclinical mastitis in lactating cows in study area (n=384).

Type of mastitis	No. of cow examined	Positive	%	Culture positive	%
Clinical mastitis	384	35	30.17	35	100
Sub-clinical mastitis	384	81	69.83	79	97.53
Total	**384**	**116**	**30.21**	**114**	**98.28**

n= number of lactating cows.

Table 2. The prevalence of mastitis in association with potential risk factors in the study areas.

Variable	Total number of examined cows	CMT positive	Prevalence (%)	X^2	P-value
Breed type					
(i) Cross breed	14 (3.65%)	10	71.43	21.9429	< 0.005
(ii) Local breed	370 (96.35%)	106	28.65		
Parity					
(i) 1-2	161 (41.93%)	21	13.04	44.82	< 0.001
(ii) 3-4	146 (38.02%)	60	41.10		
(iii) ≥5	77 (20.05%)	35	45.45		
Lactation stage					
(i) Early Lactation	130 (33.85%)	18	13.85	38.99	< 0.001
(ii) Mid Lactation	194 (50.52%)	71	36.60		
(iii) Late Lactation	60 (15.63%)	27	45.00		
Age					
(i) <4 years	135 (35.16%)	25	18.52	73.20	< 0.001
(ii) 4-8 years	167 (43.49%)	51	30.54		
(iii) >8 years	82 (21.35%)	40	48.78		

subclinical mastitis examined was higher than that reported by Alebachew and Alemu (2015) who found 21.2% clinical and 46.8% subclinical mastitis in selected commercial dairy farms in Addis Ababa. Jafer et al. (2016) also reported the minimum clinical mastitis (15.27%) and maximum subclinical mastitis (84.73%) in dairy farm of Dire Dawa City.

The present study showed an overall prevalence of 30.21% lower than that of Biffa et al. (2005) in and around Addis Ababa and Getahun (2006) in Haramaya who reported 38.9 and 36.9%, respectively. The difference in results could be due to variations in the distribution of mastitis risk, laboratory techniques, study design, climate, the level of management and animals studied. As indicted in the Table 1, all CMT positive samples were cultured for etiological agent identification. From 81 samples cultured, 79 were positive for known subclinical mastitis pathogens while all of the samples cultured from clinical mastitis were positive for mastitis.

The potential associated risk factors

Breed, parity, lactation stages and age had significant influence on the prevalence of bovine mastitis (P<0.05). The result showed that the prevalence of mastitis was significantly higher in cross breed (71.43%) than local breed of cows (28.65%) (Table 2). The effect of cross breed on the current prevalence of mastitis was relatively comparable with the reports of Jafer et al. (2016) in Dire Dawa city (71.1%). Compared to present study results, Belay and Tadele (2017) reported the lower prevalence in cross breeds (58.46%) and higher prevalence (38.2%) in local breeds in HoroGuduru Wollega Zone. In Ethiopia, many studies showed statistically significant difference in mastitis between local and cross breeds. Furthermore, cows with high milk yield is more susceptible to mastitis where as low-yielding cows tend to be more resistant (Biffa et al., 2005; Mekibib et al., 2010; Megersa et al., 2012; Moges et al., 2012). This may be due to genetic improvement for milk yield is accompanied by gradual decline in genetic resistance to mastitis (Radostits et al., 2008). Parity also showed an effect on the occurrence of mastitis. Higher prevalence (45.45%) was recorded in cows multiparous (greater than 5 parturition) and the lower prevalence (13.04%) was recorded in cows with first and second parity. Similarly, Alebachew and Alemu (2015) reported the higher prevalence (90.8%) in cows

Table 3. The identified and isolated major pathogenic bacteria species in the areas.

S/N	Isolated bacteria	Frequency	Percentage
1	*Escherichia coli*	2	2.47
2	*Staphylococcus aureus*	48	59.26
3	*Streptococcus agalactiae*	31	38.27

with 4-7 parturition and the lower prevalence (61.6%) in cows with 1-3 parturition in Addis Ababa.

Lactation stage had association with the occurrence of mastitis were the prevalence was higher (45%) in late stage, followed by middle (36.60%) and early stages of lactation (13.85%). Belay and Tadele (2017) reported that the similar results based on the stage of lactation which was 34.21, 38.24 and 56.1% in the 1st, 2nd and 3rd trimester of lactation, respectively. The high prevalence of mastitis at late lactation might be due to an increased period of exposure of the udder during previous stages of early and mid-lactations.

There was a significant difference in prevalence between animals of different age of lactating cows ($P<0.05$). The highest prevalence (48.78%) was found in lactating cows of ages greater than 8 years, followed by cows of ages 4-8 years (30.54%) and the lowest prevalence (18.52%) was recorded in cows of ages less than 4 years. Correspondingly, Belay and Tadele (2017) reported that the highest prevalence (61.16%) of older cows (>9.5 years), followed by cows age 6-9.5 years (36.96%) and the lowest prevalence (34.15%) by cows age of 2.5-6 years. The high prevalence of the mastitis revealed in older animals might be due to the physiology of exhausted canal which is more dilated and remains partially open due to years of repeated milking. This facilitates the entrance of environmental and skin-associated microorganisms leading clinical or sub clinical mastitis. Blowey and Edmondson (2010) also reported the high occurrence of mastitis in older aged cows compared to young and adult cattle. This could be due to damage of teat canals in old animals facilitates access of bacteria into the mammary gland.

Identified and isolated major pathogens

As shown in Table 3, milk samples collected from 116 mastitis positive cows (35 clinical cows and 81 CMT positive subclinical cows) were cultured on blood agar. The dominant bacteria isolated were *Staphylococcus* species followed by *Streptococcus* species and other Gram negative enteric bacteria, of which *Escherichia coli*. By using further biochemical tests and selective media, three major strains of pathogenic bacteria namely *S. aureus, S. Agalactiae* and *E. coli* were found. Among three major pathogenic bacteria, *S. aureus* was the highest prevalent organism (59.26%); followed by *S. Agalactiae* (38.27%) and *E. coli* (2.47%).

The prevalence of *S. aureus* in the present study was higher (59.26%) than early findings of Milne et al. (2002); Fufa et al. (2013) and Jafer et al. (2016) who reported 44.4% in Sebeta, 21.13% in Addis Ababa city and 48.4% in Dire Dawa, respectively. Likewise, this finding was disagreeing with the report of Bitew et al. (2010), Biruke and Shimeles (2015) who reported 20.3% in Bahir Dar and 45.1% in Addis Ababa, respectively. Present findings are comparable with the results of Endale et al. (2016) who reported 57.14% *Staphylococcus* species and 28.57% *Streptococcus* species in and around Sodo Town, Wolaita Zone, Ethiopia. The relative high prevalence of *S. aureus* in this study could be associated with the absence of post milking teat dipping, poor udder and teat washing before milking, poor hand milking practice and wide distribution of the organism inside the mammary gland and on the skin of teat and udder.

Available literature also showed that *Staphylococcus* species causing mastitis is the common and economically the greatest concern wherever dairy farming is practiced (Workineh et al., 2002; Fufa et al., 2013; Jafer et al., 2016). *S. aureus* has adopted to survive in the udder and established chronic and subclinical infection (Radostits et al., 1994). The isolation of *S. aureus* is of public health significance since it is a commonly recovered pathogen in outbreaks of food poising due to milk and milk product. This could be due to *S. aureus* is environmentally robust, surviving wide extremes of temperature and moisture. *S. agalactiae* (38.27%) was the second major pathogenic isolated in the study areas. This result was higher than the early findings of Kerro and Tareke (2003); Almaw (2004) and Bitew et al. (2010) who reported isolation rates of 13.1, 8.15 and 13.9%, respectively.

The justification given for *S. aureus* could also be a factor for *S. agalactiae* relative high isolation rate since both of them are contagious pathogens. The isolation of *streptococcus* species is of public health significance as it causes various gastrointestinal upset ranging from abdominal pain to diarrhea. Generally, the present study showed that contagious mastitis pathogens were the predominant isolated bacteria. This might be due to lack of effective udder and teat washing and drying, inter-cow hand washing and poor cleaning of milking area. Contamination of milkers' hands, cloths and milking utensils leads to high spread of mastitis disease.

The present result also indicated that *E. coli* was the third predominant pathogens (2.47%) isolated in the study areas. This finding was much lower than the early findings of Iqbal et al. (2004) and Biruke and Shimeles (2015) who reported 18.6 and 40.7%, respectively.

However, it is comparable with the previous reports of Mekibib et al. (2010) at Holeta (4.6%) and Sori et al. (2005) in and around Sebeta (0.75%). The prevalence of *E. coli* is probably due to the fact that *E. coli* is the commonest environmental contaminants which are

closely associated with hygiene. It becomes pathogenic whenever the hygienic conditions of the animal or environment become poor. In addition, the existence of high concentration of *E. coli* in milk also indicates the relatively poor quality of milk, related with substandard hygiene of the farm management.

CONCLUSION AND RECOMMENDATIONS

The overall 30.21% prevalence of mastitis at cow level was tested by using California Mastitis Test (CMT) and clinical inspection of the udder. Breed, parity, lactation stages and age have a significant influence on the prevalence of bovine mastitis ($P<0.05$). Increasing age, lactation stage, parity and poor management increased the risk of mastitis. The major pathogenic strains isolated were; *S. aureus* (59.26%), *S. agalactiae* (38.27%) and *E. coli* (2.47%). This indicates that mastitis caused by *S. aureus* is one of the major problems of dairy cows in milk production followed by *S. agalactiae*. The distribution of these bacterial pathogens in the herd indicates the economic impact of the disease. Beside the disease has economic importance it also to harm the health and well-being of human being. The professionals should apply different methods for prevention and strategic control of the disease and should be informed to the public about the relevance of pasteurization of milk before consumption to avoid food born infection and intoxication. There is a need of further study on drugs to which the bacterial are sensitive to use it used as primary choice to treat the disease in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Alebachew T, Alemu A (2015). Prevalence of bovine mastitis in lactating cows and its public health implications in selected commercial dairy farms of Addis Ababa. Global Journal of Medical Research: Global Veterinary Science and Veterinary Medicine 15(2):16-24.

Almaw G (2004). A cross-sectional study of bovine mastitis in and around Bahir Dar and antibiotic resistance patterns of major pathogens. Addis Ababa University, Faculty of Veterinary Medicine DVM Thesis.

Almaw G, Molla W and Melaku A (2009). Prevalence of bovine subclinical mastitis in Gondar town and surrounding areas, Ethiopia. Livestock Research Rural Development 21(7).

Belay B, Tadele T. (2017). Epidemiology and financial impact of bovine mastitis in an animal production and research center and small holder dairy farms in Horo Guduru Wollega Zone, Western Ethiopia. Journal of Dairy Veterinary and Animal Research 5(4):8-9.

Biffa D, Debela E, Beyene F (2005). Prevalence and risk factors of mastitis in lactating dairy cows in southern Ethiopia. International. Journal of Applied Research in Veterinary Medicine 3(3):189-198.

Biruke D, Shimeles A (2015). Isolation and identification of major bacterial pathogen from clinical mastitis cow raw milk in Addis Ababa, Ethiopia. Academic Journal of Animal Diseases 4(1):44-51.

Bitew M, Tefera A, Toles T (2010). Study on bovine mastitis in dairy farms of Bahir Dar town and its environs. Journal of Veterinary and Animal Advances 9:2912-2917.

Blowey R, Edmondson P (2010). Mastitis Control in Dairy Herds (2^{nd}edn) CAB. International, UK.

Central Statistical Agency (CSA) (2015). Report on livestock and livestock characteristics (private peasant holdings). Agricultural Sample Survey. Volume V. Addis Ababa, Ethiopia pp.109-126.

Central Statistical Agency (CSA) (2016/17). Report on livestock and livestock characteristics (private peasant holdings), Agricultural Sample Survey, Volume II. Addis Ababa, Ethiopia.

Endale M, Eyob E, Addisu A, Naod T (2016). A Study on the prevalence of bovine mastitis and associated risk factors in and the surrounding areas of Sodo Town, Wolaita Zone, Ethiopia. Global Journal of Medical Research: Global Veterinary Science and Veterinary Medicine 16(2):16-24.

Food and Agricultural Organization of the United Nations (FAO) (2017). Livestock production systems spotlight cattle sectors in Ethiopia. Africa Sustainable Livestock 2050. Available at: http://www.fao.org/ag/againfo/programmes/en/ASL2050.html

Food Hygiene Regulations (2006). A practical guide for milk producers to the food hygiene regulations, England 6 p.

Fufa A, Gemechis F, Bekele M, Alemayehu R (2013). Bovine mastitis: prevalence, risk factors and bacterial isolation in small-holder dairy farms in Addis Ababa City, Ethiopia. Global Veterinaria 10(6):647-652.

Getahun K (2006). Bovine mastitis and antibiotic resistance patterns of major pathogens in smallholder dairy forms in central high land of Ethiopia. MSc thesis: Debre-Zeit: Factory of Veterinary medicine, Addis Ababa university, Ethiopia.

Iqbal M, Khan MA, Daraz B, Saddique U (2004). Bacteriology of mastitic milk and in vitro antibiogram of the isolates. Pakistan Veterinary Journal 24:161-164.

Jafer K, Haimanot D, Hawi J, Tilahun Z, Girma K (2016). A Study on bovine mastitis, isolation and identification of staphylococcus species in Dairy Farms of Dire Dawa City, Eastern Ethiopia. Global Veterinaria 16(3):222-230.

Kerro OD, Tareke F (2003). Bovine mastitis in selected areas of Southern Ethiopia. Tropical Animal Health Production 35:197-205.

Lakew M, Tolosa T, Tigre W (2009). Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. Tropical Animal Health and Production 41:1525-1530.

Lemma M, Kassa T, Tegegene A (2001). Clinically manifested major health problems of crossbred dairy herds in urban and peri-urban production systems in the central high lands of Ethiopia. Tropical Animal Health and Production 33:85-89.

Megersa B, Manedo A, Abera M, Regassa A, Abunna F (2012). Mastitis in lactating cows at Hawassa town: prevalence, risk factors, majorbacterial causes and treatment response to routinely used antibiotics. American-Eurasian Journal of Scientific Research 7(2):86-91.

Mekibib B, Furgassa M, Abuna F, Megersa B, Regassa A (2010). Bovine mastitis: prevalence, risk factors and major pathogens in dairy farms of Holleta town, Central Ethiopia. Veterinary World 9(3):397-403.

Mekonnen H, Workineh S, Bayleyegne M, Moges A, Tadele K (2005). Antimicrobial susceptibility profile of mastitis isolates from cows in three major Ethiopian dairies. Medicine Veterinary 176(7):391-394.
Milne MH, Barrett DC, Fitzpatrick JL, Biggs AM (2002). Prevalence and an etiology of clinical mastitis on dairy farms in Devon. Veterinary Record 158:241-243.
Moges N, Hailemariam T, Fentahun T, Chanie, Melak A (2012). Bovine mastitis and associated risk factors in small holder lactating dairy farms in Hawassa, Southern Ethiopia. Global Veterinaria 9(4):441-446.
National Mastitis Council (NMC) (1990). Microbiological procedures for the diagnosis of bovine udder infection.3rd. Arlington, Va, USA: National Mastitis Council Inc.
Oviedo-Boyso J, Valdez A, Cajero-Juárez M, Ochoa-Zarzosa A, Meza-López-Meza JE, Patino A, Baizabal-Aguirre VM (2007). Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. Journal Infection 54:399-409.
Quinn PJ, Carter ME, Markey B Carter GR (1994). Clinical veterinary microbiology, Wilfe Publishing, London pp. 95-101.
Quinn PJ, Carter ME, Markey B, Carter GR (1999). Clinical veterinary microbiology. Harcourt Publishers Ltd.: London.
Quinn PBK, Markey ME, Carter, Donnelly WJC, Leonard FC and Maguire D (2002). Veterinary microbiology and microbial diseases.1st Published Blackwell Science 1td. Radostits OM, Blood DC, Gay CC (1994). Veterinary Medicine: A text book of the diseases of cattle, sheep, pigs, goats and horses. 8th ed. Bailliere Tindall: London 8:563-613.
Radostits OM, Blood DC, Gay CC (1994). Veterinary Medicine: A text book of the diseases of cattle, sheep, pigs, goats and horses. 8th ed. Bailliere Tindall: London 8:563-613.
Radostits OM, Gay CC, Blood DC, Hinchlif KW (2007). Mastitis In: Veterinary medicine 9thed., Harcourt Ltd, London pp. 174-758.
Radostits OM, Gay GC, Blood DC, Hinchillif KW (2000). Mastitis In: Veterinary Medicine, 9th Edition, Harcourt Limited, London pp. 603-700.
Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2008).Veterinary Medicine; Text books of the disease of Cattle, Sheep, Pigs, goats and Horses.(10thedn), Elsevier, UK. pp. 673-680.
Seegers H, Fourichon C, Beaudeau F (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. Veterinary Research 34(5):475-491.
Sori H, Zerihun A, Abdicho S (2005). Dairy Cattle Mastitis In and Around Sebeta, Ethiopia International Journal of Applied Research in Veterinary Medicine 3:332-338.
Suriyasathaporn W, Schukken YH, Nielsen M, Brand A (2000). Low somatic cell count: a risk factor for subsequent clinical mastitis in dairy herd. Journal of Dairy Science 83:1248-1255.
Teshome G, Tesfaye A (2016). Physicochemical properties and microbial quality of raw cow milk produced by smallholders in Bench Maji-Zone, Southwestern Ethiopia. Food Science and Quality Management 55:55-62.
Thrusfield M (2007). Veterinary epidemiology 3rd edition, Blackwell Publishing.
Workineh S, Bayleyegn M, Mekonnen H, Potgieter LND (2002). Prevalence and an etiology of mastitis in cows from two major Ethiopian dairies. Tropical Animal Health and Production 34:19-25.

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Full Length Research Paper

Common health and welfare problems of working donkeys in Addis Ababa and its surrounding area: Retrospective and questionnaire survey

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A retrospective study was conducted to assess the health and welfare problems of working donkeys at the Merkato Donkey Sanctuary clinic in Addis Ababa, Ethiopia. Data on 12,991 working donkeys from 2008 to 2010 were analyzed from the data record sheet kept by the clinic. Results on the age distribution of donkeys showed that the average age of the donkeys was around nine years old. Only 7.37% of the donkeys were with good or ideal body condition (≤2.5), while the majority, 90.56% were with poor body condition (≤2). Multiple health problems were a common feature. Among the common health problems encountered parasitosis, hoof problems, wounds, musculoskeletal disorders, colic, ophthalmic cases and traffic accidents accounted for 55.68, 16.71, 14.9, 3.28, 2.59, 2.37, and 1.71%, respectively. Wounds due to ill fitted harnesses and inappropriate harnessing materials accounted for majority of the wounds observed. Back sore due to the absence of paddings, improper padding, inadequate and inappropriate padding materials was a common feature, and accounted for 62.6% of the overall different wounds. Hoof overgrowth and solar puncture and/or abscess due to sharp objects were the common hoof problems recorded, while hoof overgrowth accounts for 13.56%. Traffic accidents leading to death and injuries were common problems. Apart from the health related welfare problems, the questionnaire survey made also revealed that abuses and negligence by the owners, management constraints like overloading and overworking, beating, and shortage of feed, housing problems, wounds due to harnessing and physical injuries mainly due to traffic accidents were the major welfare problems of urban working donkeys. The retrospective study, the questionnaire survey and observations made provided the significant health and welfare problems of working donkeys that need to be addressed in order to improve their health and working efficiency.

Key words: Retrospective study; working donkey; health and welfare problems.

INTRODUCTION

World domesticated equines (horses, donkeys, and mules) population is 115.2 million consisting of 44.3 million donkeys. Global distribution indicated that 98% of all donkeys are found in developing countries (Jannke, 1983). Ethiopia having5.2 million donkeys is the second in donkey population in the world and first in Africa

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possessing nearly 40% of African donkey population. According to the present regional classification of Ethiopia 97% of donkey population is found in three regions: 44% in Oromia, 34% in Amahara and 19% in Tigray regional state (Feseha, 1998).

In Ethiopia they have been used as beasts of burden for a long time and still render their valuable services mostly as pack animals throughout the country, particularly in areas where modern means of transportation are absent, unaffordable or inaccessible (Getachew et al., 2002). Donkeys have been completely neglected and omitted from the national livestock development programs. This is because the contribution of donkey's power in the agricultural systems and their role in production are not yet well recognized and magnified (Fielding, 1987). The treatment services provided to these species of animals have been far below that is given to other species of animals; this can be due to age old erroneous concept that when donkeys do get sick they are quick to die and probably because they are not provider of meat and milk (Yoseph et al., 2001).

Due to minimum management attention is given to donkeys, particularly in countries like Ethiopia; they are exposed to some diseases. Donkeys in Ethiopia are subjected to numerous health and welfare problems. These include: polyparasistism, back sores, and other harness inflicted wounds, hoof problems, ophthalmic problems, colic, car accident, overloading and overworking, and various infectious diseases such as strangles and Tetanus, Donkey Sanctuary-Donkey Health and Welfare project (DS-DHWP) in Ethiopia has been providing veterinary, education and extension services since its inception in 1994. It opened its second stationary clinic in December 2007 in Addis Ababa, Merkato grain market, where more than 5000 donkeys are giving their pack transport services to the urban communities. The clinic provides veterinary services to many health and management problems of working donkeys. The health and welfare problems of these working donkeys are not well documented and readily available. Therefore, the primary objective of the present study was to identify the common health, welfare, and management problems of working donkeys in Addis Ababa-Ethiopia, Merkato grain market.

MATERIALS AND METHODS

Study area

The study was conducted in Addis Ababa, Merkato Donkey Sanctuary (DS) clinic. Donkeys are coming to get treatments from within Addis Ababa and the surrounding areas of Sululta and Gefersa. Merkato is known for its high donkey population used by limited resource communities for their livelihood.

Addis Ababa- located at 9°2'N', 38042'E with an altitude of about 2400 m above sea level, Addis Ababa city possesses a complex mix of highland climates zones with temperature differences of up to 10°C depending of elevation and prevailing wind patterns, high elevation moderate temperature year round, and the city's position near the equator mean that temperatures are constant from month to month. Addis Ababa receives a mean annual rainfall of 1800 mm in bimodal pattern. The long rainy season extends from June to September followed by a dry season ranging from October to February; the short rainy season lasts from March to May. The average minimum and maximum temperature of Addis Ababa is 10.7 and 23.6°C, respectively (NMSA, 2005).

Study animals

The study animals were working donkeys in Addis Ababa, and the surrounding areas of Sululta and Gefersa coming to the Merkato DS clinic for treatment against different health problems. Most of the donkeys were from the Merkato grain market and some from the different sub-cities of Addis Ababa.

Study design

Retrospective studies

Data on the different health, management and welfare problems of working donkeys that came to the Merkato DS clinic for treatment were stored either electronically or on a data record sheet as a hard copy since 2008. Data from daily clinical record formats/sheet were entered into Microsoft Excel spread sheet. The data were coded into categories of clinical findings for each donkey. The categories were defined according to the systems affected, types of the problem and the cause of the problems. A total of 12,991 records of donkeys from 2008 to 2010 were extracted and organized for further analysis.

Questionnaire survey

A structured questionnaire was designed and validated to cover a wide range of socio-economic aspects including the number of donkeys owned, size at house hold levels, uses, family income through the use of donkeys, frequency and magnitude of work, nutrition and management of donkeys, health and welfare constraints and significant causes of abandoning working donkeys. The questionnaire was randomly administered to donkey owners, drivers coming to the Merkato clinic to collect relevant information about working donkey's welfare issues in the study area. For the purpose, a total of 71 donkey owners were interviewed.

Data analysis

Descriptive statistics for the common health problems of working donkeys were calculated using Minitab statistical software. Graphs and tables were produced using Microsoft Excel program.

RESULTS

Retrospective study

The data record format showed that some donkeys were coming to the clinic to get treatments against different health problems (Figure 1). The study made showed that 96.3% of the donkeys in and around Addis Ababa used for work were adults greater than four years old. However, more than 3% of the donkeys were less than three years of age. The average age of the donkeys was

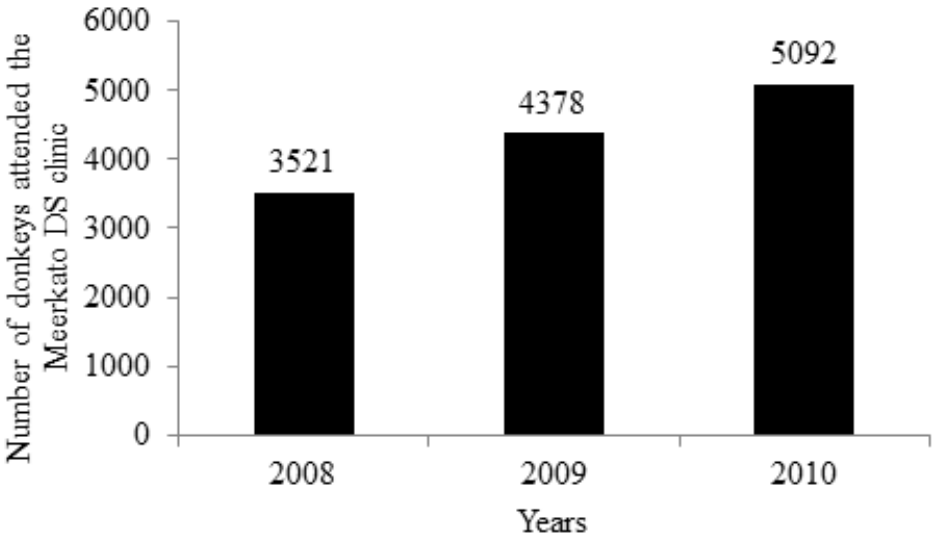

Figure 1. Number of donkeys got treatment at the Merkato DS clinic during the year 2008-2010.

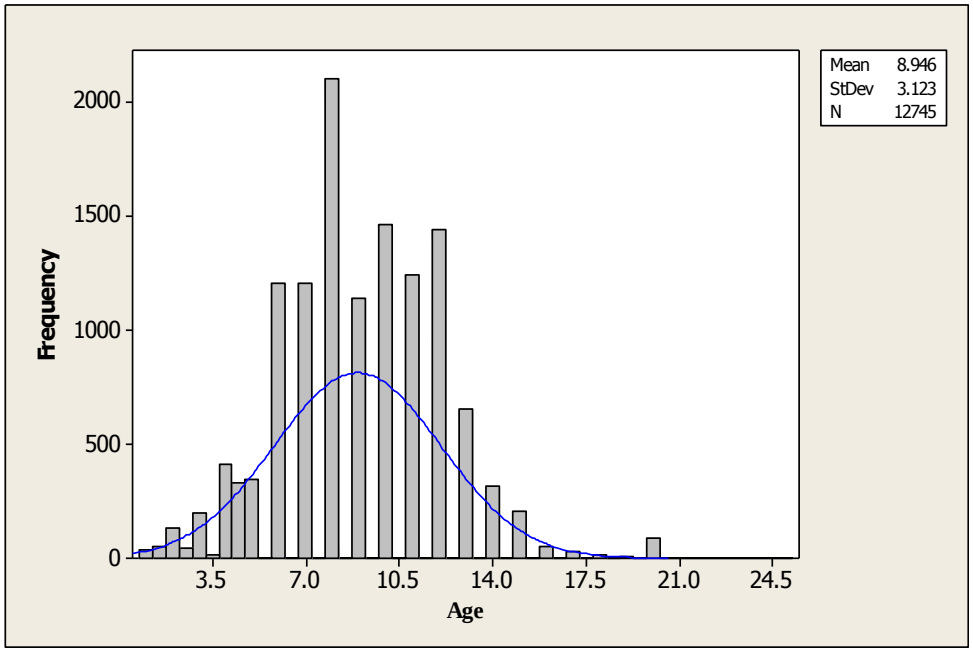

Figure 2. Frequency distribution of the age of working donkeys treated at Merkato DS clinic in Addis Ababa during the year 2008-2010, Ethiopia.

nine years old. Age distribution of the donkeys is shown in Figure 2. The frequency distribution of the body condition score of the donkeys is shown in Figure 3. More than 90% of the donkeys were with poor body condition score (≤2), while it is only 7% of the donkeys had well to ideal (≥2.5) body condition. Further analysis of the data revealed that donkeys were suffering from multiple health and welfare problems. The common health problems of working donkeys are summarized in Table 1.

Parasitosis, Wounds, hoof problems, musculoskeletal disorders, colic, ophthalmic problems and traffic accidents were the major problems encountered in working donkeys during the year 2008-2010 (Table 1). Wounds due to the use of inappropriate harnessing materials, absences of padding for the back and ill fitted harnessing materials were the primary cause of wounds and sores. The most common wound was back sore (Figure 4). Among hoof problems, hoof overgrowth and

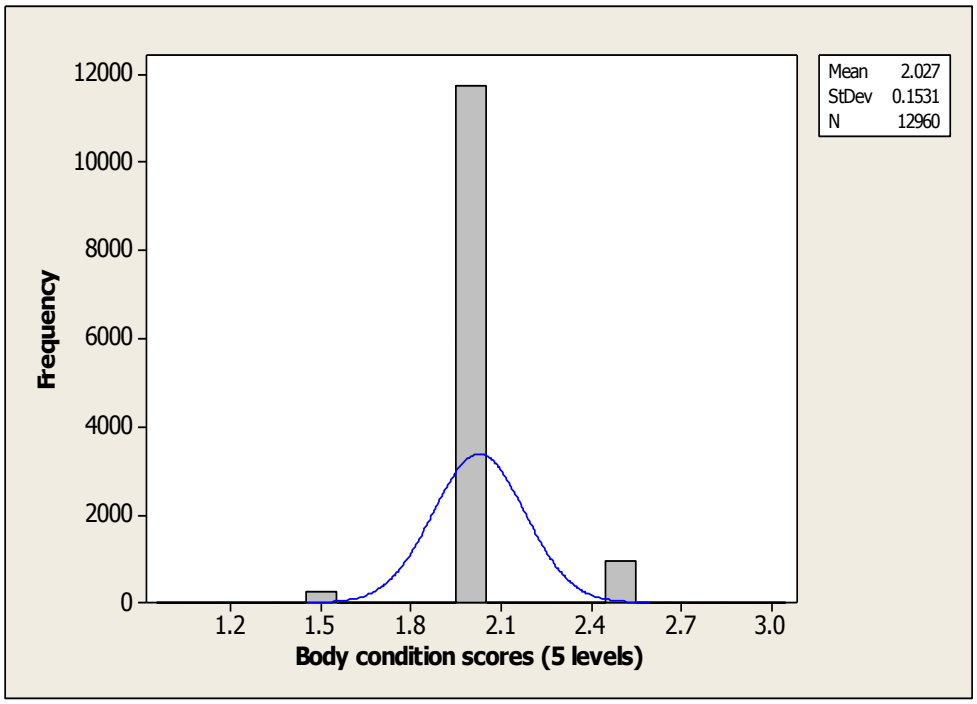

Figure 3. Frequency distribution of body condition of working donkeys treated at the Merkato DS clinic in Addis Ababa during the year 2008-2010, Ethiopia.

Table 1. Common health problems of working donkeys diagnosed and treated at Merkato DS clinic in Addis Ababa (n=12,991).

Cases attended	Number of cases	Percentage
Wounds		
Back sores	1195	9.2
Other wounds	734	5.7
Total	**1929**	**14.9**
Colic		
Due to Foreign bodies	154	1.2
Parasitic	38	0.29
Impaction	10	0.08
Unknown causes	132	1.02
Total	**338**	**2.59**
Hoof problems		
Hoof overgrowth	1762	13.56
Solar puncture/abscess	268	2.08
Hoof thrush	141	1.09
Total	**2171**	**16.71**
Musculoskeletal disorders		
Mechanical injury	41	0.32
Muscular strain	385	2.96
Total	**426**	**3.28**
Ophthalmic cases		
Mechanical injury	222	1.71

Table 1. Contd.

Unknown cause	86	0.66
Total	**308**	**2.37**
Respiratory problems		
Drenching pneumonia	18	0.14
Suspected strangles	9	0.07
GIT Parasites	59	0.45
Total	**86**	**0.66**
Parasitosis	7234	55.68
Traffic accidents	183	1.41
Dental problems	89	0.69
Infectious cases	49	0.38
Tumours	55	0.42
Rectal prolapse	23	0.18
Suspected anthrax	30	0.23
Suspected rabies	11	0.08
Tetanus	10	0.08
Miscellaneous	35	0.27

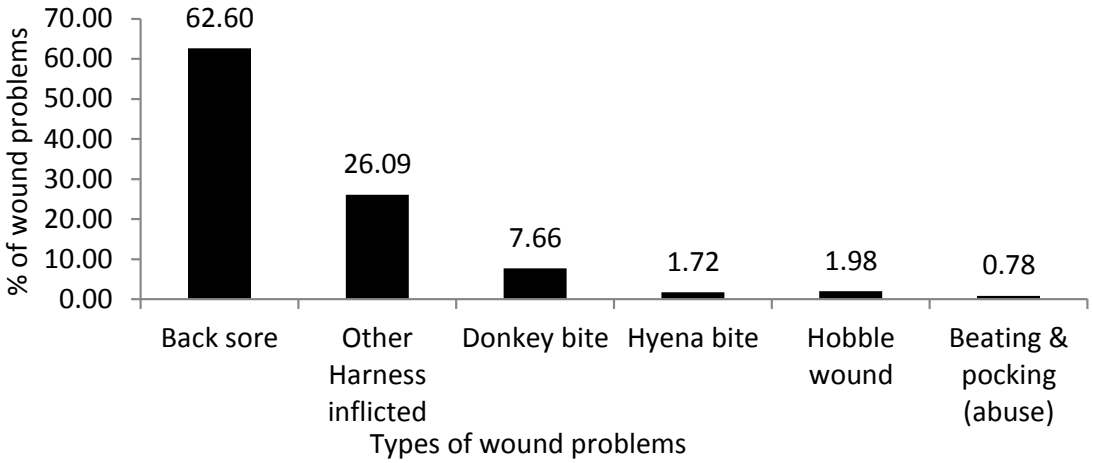

Figure 4. Common wound problems of working donkeys treated at the Merkato DS clinic during the years 2008-2010 (n=1945).

puncture wounds leading to solar abscess and lameness were common (Figure 5).

Questionnaire survey

Ownership, owners' education back ground and working condition of donkeys:

Questionnaire survey showed that 83% of the owners get 60-100% of their income from the work of their donkeys. Over 90% of the donkey owners were more than 25 years old ranging from 15-85 years. Education background of the donkey owners showed 56% were illiterate and only 31 and 14% attended elementary and secondary school level, respectively. Ownership of the donkeys showed that more than 83% had 2 to 3 donkeys, and the average number of donkeys per owner was 3. Eighty seven percent of the owners reported that the average weight they load their donkeys was 85 kg (50-130 kg), travelling 7 h per day covering an average distance of 20 km. Seventy six percent of the owners use

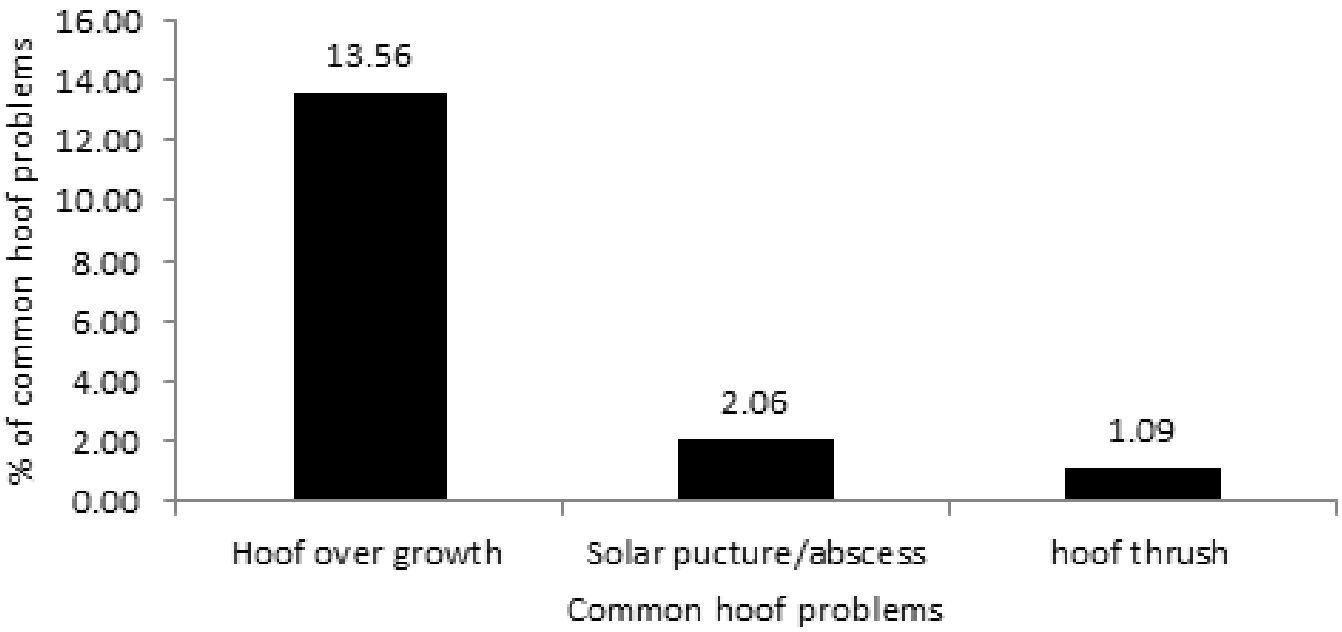

Figure 5. Common hoof problems of working donkeys treated at the Merkato DS clinic during the year 2009-2010 (n= 2171).

their donkeys 2-5 days per week covering a distance of 2.5-35 km.

Water, feed, feeding and housing practices

Almost all donkeys in urban areas depend on hand feeding since grazing is quite scarce. Over 83% of the owners reported that they supplement their animals with 'kortebe', the local name for a mixture of different cereals/grain leftovers while other provides grains and straws/grass. Over 66% of the owners reported that they feed their donkey only once a day, while 33% feed twice a day. Majority of the owners provide water twice a day. Most of the donkeys spend day time working, and have a limited time roaming around and/or grazing, if available. Over 91% of the owners reported that donkeys are housed at night separately in purposely built enclosures.

Health and management problems

Ninety five percent of the owners reported parasitosis as a significant disease problem incriminated in reducing the working performance of their donkeys. Hoof problems and wound/traumatic injuries were ranked second and third major essential health problems, respectively. The owners do not practice castration of male donkeys because they believe that castration reduces working capacity and life span of their donkeys. From the interviews and observation made, health care given to the animals is negligible and more than 70% of the owners practice traditional methods of treatment when their donkeys get sick. Drenching herbal plants mixed with some spices and alcohol (Areke), the use of engine oil for wound treatment, and branding for lameness cases related to musculoskeletal problems are common practices.

DISCUSSION

The present retrospective study has revealed multiple health, management and welfare problems of working donkeys in and around Addis Ababa. Although the majority of the donkeys were adults, over four years of age, the 3% of donkeys below three years of age indicate that donkey owners begin to use donkeys for work before they are mature enough. Age at maturity of donkeys is estimated at four years and it is recommended not to work with them until this age as this predisposes them to structural deformities such as sagged (lordosis) back and early demise. Although good to ideal body condition was observed in 7% of the population, the majority of the donkeys were with poor body condition (bcs<2). Donkeys studied were, on the whole, in poor body condition with a mean/median body condition of 2. This clearly shows that apart from health and management related problems, shortage of feed may be a major contributory factor for poor body condition of the donkeys the result obtained by a questionnaire survey, they have low priority regarding access to good quality feed. Pearson et al. (2001), similar finding. Such poor body condition might be one of the contributory factors for the high incidence of back sore, due to less muscle cover.

A large proportion of donkeys were seen with various degrees of wounds and abrasions. These results were consistent with the finding of similar works done by Mohammed (1991), Pearson et al. (2001) Demelash and Moges (2006) in Ethiopia, Rodriguez-Maldonado (1990) in Mexico, Harris (1971) in UK. The major causes of wounds and injuries were harness related problems due to inappropriate harnessing material, ill-fitted harnesses and the absence of padding on the back of the animals. Abnormalities in locomotion incident to joint sprains and unknown causes suggested that donkeys were overloaded, leading to tendon damage.

The observed high incidence of hoof problems could have resulted from lack of proper hoof trimming and care due to the lack of veterinary services (Soliman, 1989). Similar hoof problems were reported by Getachew et al. (2002a) and Yilma et al. (1991). The finding of questionnaire survey also reflects this problem, the high incidence of hoof overgrowth and lameness in the present study is not consistent with the findings of Getachew et al. (2002) in rural donkeys. The travelling of donkeys in a narrow lane in the city might have predisposed them to hoof puncture with sharp objects such as nails and broken glasses, which are quite common in such urban areas. Traffic accidents also played a significant role in causing hoof problems leading to lameness. According to the study made by Morgan (2006), lameness related to foot problems due to traffic trauma was a common problem in urban donkeys that is consistent with current finding.

Wounds from donkey bites are results of fighting between males donkey, as the practice of castrating donkey is not known in the areas, and this may lead to aggressive behavior to each other. The low incidence of hobble wounds in this study indicates that hobbling donkeys is not a common practice in urban donkeys as compared to rural donkeys, which is one of the common causes of injury and abrasion (Feseha, 1997; Getachew et al., 2002b). Both the retrospective study and questionnaire survey showed that traffic accidents are quite common in urban working donkeys. The present 1.4% traffic accident cases were those lucky ones with treatable injuries and able to recover. However, according to the clinician and our observation, the majority of traffic accidents result in death and serious structural damage, which necessitate euthanasia. Study made by Getachew et al. (2002) reported traffic accident as one of the major problems in working donkeys living in the urban setups and by the road sides, which frequently cross roads on their way to graze or home. The fact that donkeys carrying heavy loads and sharing the same road in the urban setup with vehicles predisposes them to the accident.

Colic cases due to the ingestion of a foreign body, particularly plastic materials were found to be one of the significant gastrointestinal problems, which are consistent with the finding of Getachew et al. (2002). Apart from ingestion of foreign bodies, impaction due to ingestion of excessive coarse, dry and high fiber feeds such as the fine residue of teff (*Eragositis abyssinica*) is a frequently encounter cases of colic in rural donkeys during the harvesting and threshing seasons (Getachew, et al., 2002).

The present finding of respiratory problems is consistent with the reports by Feseha (1997), Pearson et al. (2001) and Getachew et al. (2002). Majority of the respiratory problems were due to drenching of herbal remedies to treat other problems, particularly colic cases. The common use of traditional herbal remedies from the reflection of those. The finding of cases of rabies, anthrax, tetanus, strangles, rectal prolapse and tumour in this study was consistent with the results of the study made by Getachew (1999), Pearson et al. (2002), Getachew et al. (2002) and Ayele et al. (2006).

CONCLUSION AND RECOMMENDATIONS

The present retrospective study and questionnaire survey revealed that there are a significant health, management and welfare problems of working donkeys in the urban and per-urban setup of Addis Ababa. The study has shown that majority of the problems were due to mismanagement, neglect and cruelty which could easily be preventable, however, deliberate maltreatment is rare and health problems of donkeys are more likely due to ignorance. The ubiquitous nature of the problems may result in donkey owners becoming indifferent or being unaware that anything is wrong. The main reasons for the mismanagement and ill-treatment of donkeys could be many folds:

(i) The weak economy of the owners
(ii) Lack of education and training
(iii) Lack of material essential drugs
(iv) Lack of professional advice
(v) The perception by the people that donkeys do not get ill or can tolerate problems may also play a significant role.
(vi) Fundamental lack of understanding of the potential productivity of their animals giving the correct care by the owners.

The health, management and welfare problems of donkeys can be improved and controlled, and the increased use of donkeys can be enhanced:

(vii) Proper veterinary care and advice
(viii) Through the development and dissemination of proper harnesses to control wounds and sores.
(ix) Through better education and training of both professionals and donkey owners as to the primary health care, management and welfare problems of donkeys.
(x) When the donkey is seen by the whole community as an animal for collective benefit, and not just for the

individual, who owns them.
(xi) When a better awareness of its utility and its possibility as an economic force is recognized by the people concerned with developmental programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Ayele G, Feseha G, Bojia E, Getachew M, Alemayehu F, Tesfaye M, Amare B, Dereje N, Chala C, Asefa A, Anzuino G, Trawford A (2006). Sarcoids: clinical epidemiology, principal effect and treatment responses. Proceedings of the fifth international colloquium on working equines. 30th Oct-2nd Nov, 2006, Addis Ababa, Ethiopia pp. 90-106.

Ayele G, Feseha G, Bojia E, Joe A (2006). Prevalence of gastro intestinal parasites of donkeys in Dugda district, Ethiopia, Livestock Research for Rural Development 18:2-6.

Demelash B, Moges W (2006). Causes in draught and factors associated with occurrence of external injuries in working equines in Ethiopia. International Journal of Applied Research in Veterinary Medicine 4(1):7.

Feseha G (1998). Maximizing the use of donkey in the dry lands of Ethiopia; producing of the national work shop on challenges and opportunities for research and agro pastoral areas of Ethiopia, 16-18 December 1998, Mekelle, Ethiopia.

Feseha, G. (1997). Diseases and health problems of donkeys abroad. In: Svendsen, E.D (ed). The professional Handbook of the donkey, 3rd edn. Whittet Books Ltd. London, UK. pp. 207-226.

Fielding D (1987). Donkey power in Africa Rulal transport world. Annual. Review 63:22-30.

Geatchew M (1999): Epidemiological study on the health and welfare of the Ethiopian donkey, with particular reference to parasitic diseases. MVM thesis, University of Glasgow, UK.

Getachew M, Feshea G, Andrew FT, Bojia E, Alemayehu F, Amare B (2002). Major diseases problems of donkeys. In; pearson, A., finding, D., Jabbaa, D.Fourth international collaguium on working Equines. SPANA-AL Baath University, Hama, Syria, 20th – 26th April, pp. 96-109.

Getachew M, Feseha G, Trawford A, Bojia E, Alemayehu F, Amare B, (2002). Some common clinical cases and intervention made at the donkey health and welfare project-Donkey sanctuary, Debre Zeit, Ethiopia. Proceddings of the 4th International Colloquium on working equines. 20-26th April, 2002. Hama, Syria. pp. 96-109.

Harris DG (1971). Mules and Donkeys. In: Universities of Farm Animals Welfare (UFAW), hand book on the care and management of farm animals. Blackwell publishers, UK. pp. 194-195.

Jannke HE (1983). Feeding and care of Horse Churchill Livestock Development in Tropical Africa Kiel Kieler, wissen schaff svarlag vult.

Mohammed A (1991). Management and breeding aspects of donkeys around Awassa, Ethiopia, In: Fielding D and Pearson R A (Editors). Donkeys, Mules and Hores inTropical Agicultural Development pp 185-188. CTVM: Edinburgh UK.

Morgan R (2006). The epidemiology of lameness in working donkeys in Addis Ababa and the central Oromia region of Ethiopia: a comparative study of urban and rural donkey populations. Proceeding of the fifth international colloquium on working equines. 30th Oct-2nd Nov, 2006, Addis Ababa, Ethiopia pp. 99-109.

National Metrology Service Agency (NMSA) (2005). Addis Ababa, Ethiopia. (http://www.wamis.org/countries/ethiopia/Eth20051503.pdf)

Pearson RA, Mengistu A, Agajie T, Allan EF, Smith DG, Mesfin A, (2001). Use and management of donkeys in per-urban areas of Ethiopian. Report of phase one of the CTVM/EARO Collaborative project April 1999-June 2000.

Rodriguez-Maldonado G (1990). The principle problems of working donkeys in Mexico. In: Fielding D and Pearson R A (eds), Donkeys Mules and Horses in Tropical Agricultural Development. Centre for Tropical Veterinary Medicine, University of Edinburgh, Edinburgh pp. 138-139.

Soliman IA (1989). Clinical observations of hoof deformities in donkeys. Assiut Veterinary Medical Journal 21:163.

Yilma J, Fesseha G, Sevendsen ED, Mohammed A (1991). Health problems of Working Donkeys in Debre Zeit and Menagesha Rgions of Ethiopia. In: finding D and pearson A (ed): Donkeys, mules and horses in tropical agricultural development proceedings of a cllaguium held 3-6 September 1990, scot land. Centre for Tropical Veterinary Medicine, University of Edinburgn pp. 151-155.

Yoseph S, feseher G, Abebe W (2001): Survey of halmintnosis of equines in Wonchi Ethiopia. Journal of the Ethiopian Veterinary Association 5(1):47-61.

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Distribution and molecular characterization of avian hepatitis E virus (aHEV) in domestic and wild birds in Burkina Faso

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Avian hepatitis E virus (aHEV), clinically important in poultry industry, can cause death and reduce egg production of chickens, resulting in significant economic losses in the poultry industry. However, little is known about this aHEV infection in Burkina Faso. This study presents the results of distribution and characterization of aHEV in domestic and wild birds without clinical disease. In total 173 birds liver samples were collected from four Burkina Faso provinces, between February 2015 and June 2016. Reverse transcription polymerase chain reaction (RT-PCR) with aHEV specific degenerate primers was used to screen the presence of aHEV. RNA of aHEV was detected in 29 (16.8%) liver samples. Of these, the prevalence was diverse in different species of birds; the most frequent level was 35.3% in *Numida meleagris*, respectively followed by 23.5% in *Gallus gallus domesticus*, 13.3% in *Streptopelia turtur*, 13.3% in *Columba livia*, 6.7% in *Anas platyrhynchos* and 3.3% in *Pternistis natalensis*. The present study firstly revealed the prevalence of HEV infection in six species of birds in Burkina. It is therefore important to conduct further research on the impact on poultry mortality and egg production in our country.

Key words: Avian hepatitis E virus, zoonosis, birds, prevalence, Burkina Faso.

INTRODUCTION

Hepatitis E virus (HEV), known to have zoonotic potential (Pavio et al., 2010), is transmitted enterically, mainly

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through the consumption of contaminated food or water (Yugo and Meng, 2013). HEV is the causative agent of a self-limiting acute hepatitis, ranges from an asymptomatic to a severe course, as described in immune-compromised patients and pregnant women (Purdy and Sue, 2017; Zuin et al., 2017). The severity in pregnant women is reflected in a mortality rate reaching up to 10 to 30% compared with 0.5 to 4.0% in young adults (Ward et al., 2011). HEV is divided into two genera: *Orthohepevirus* with four species (A–D) and *Piscihepevirus* with one species (Spahr et al., 2018). *Orthohepevirus A* has at least 8 recognized genotypes of mammalian HEV. *Orthohepevirus B* consists of avian viruses and is divided into four proposed subtypes (I–IV) associated with geographical distribution (Sridhar et al., 2017).

Avian Hepatitis E virus (aHEV) was first isolated from chickens with big liver and spleen disease (BLSD) or hepatitis-splenomegaly (HS) syndrome. Phylogenetic analysis of the full or nearly complete genome of aHEV strains identified four different genotypes and showed a distant relationship to mammalian and swine HEVs (50 to 60% nucleotide sequence identity) (Smith et al., 2015). The aHEV genotype 1 has been described in Australia and Korea, genotype 2 in USA, genotype 3 in Europe and China, and more recently, genotype 4 in Hungary and Taiwan (Payne et al., 1999; Park et al., 2015; Wang et al., 2015; Moon et al., 2016; Zhang et al., 2016).

HEV infections are completely asymptomatic in many animal species, but it seems to have some pathogenic importance for chickens (Yugo et al., 2016). Besides the enlargement of spleen and liver, both ovarian regression and presence of serosanguinous abdominal fluid or clotted blood in the abdomen are commonly associated with the HS syndrome (Ritchie and Riddell, 1991; Payne et al., 1999; Haqshenas et al., 2001; Thiry et al., 2017). The disease mainly causes a decrease in egg production and an increase in mortality in birds (Sun et al., 2004; Peralta et al., 2009). However, aHEV can be detected in birds without symptoms as well (Yugo et al., 2016; Zhang et al., 2016; Zhang et al., 2017). The virus appears to spread easily within and between flocks via the fecal-oral route transmission (Yugo et al., 2016). Other routes of transmission, including aerosol, vertical, vector-borne, or mechanical carrier, have not been demonstrated in natural or experimental avian models (Meng, 2011).

Based on serological evidence, it appears that avian HEV is widespread in chicken flocks with seropositive rates of approximately 71% in the United States, 90% in Spain, 20% in Brazil and 57% in Korea (Kwon et al., 2012). The overall detection rate of avian HEV RNA in fecal samples was 62.9% in the United States (Gerber et al., 2015).

Human infection with aHEV has not been observed up to now as it was for swine HEV (Meng, 2010). However, aHEV exposure of human population have largely increase in relationship to the consumption of contaminated poultry eggs and meat, the use of poultry viscera as a culinary delicacy, and the handling of poultry (Hsu and Tsai, 2014). In addition to the already described capacity of the virus to recognize human hepatocyte (Hsu and Tsai, 2014), the existence of a yet unknown aHEV variant able to enter and infect human liver may have a critical public health implication in the future.

In West Africa, the status of avian HEV infection in chickens is largely unknown. Considering that aHEV infection is most prevalent and dangerous among birds, it is imperative to access the contribution of aHEV to poultry and wildlife in Burkina Faso. The aim of the present study were to determine the possible circulation of avian HEV both in domestic and wild birds without clinical symptoms in Burkina Faso.

MATERIALS AND METHODS

Sample collection

In total, 173 samples of different symptomless bird flocks (4 domestic bird species) or hunted animals (2 wild bird species) currently in food chain in Burkina, were collected between February 2015 and June 2016 from four Burkina Faso district (Figure 1): 34 Guinea fowls (*Numida meleagris*), 34 chicken (*Gallus gallus domesticus*), 30 mallards (*Anas platyrhynchos*), and 15 doves (*Columba livia*) for domestic flocks and 30 turtle dove (*Streptopelia turtur*), 30 natal francolins (*Pternistis natalensis*) hunted in the hunting areas of Burkina Faso.

0.5 g of liver samples from each animal were collected and stored at -20°C in the RNAlater Buffer, until further use as source of HEV genomic RNA. Wild animals were samples in the provinces of Houet and Gourma where there are hunting areas. Kadiogo is in the center and does not have a hunting area, so no wild birds were taken in this area.

Samples RNA extraction and aHEV detection

RNA extractions on the liver samples were performed using the SV total RNA isolation system kit (Promega, France). Extracts were subsequently used for detection of the partial capsid gene of aHEV using primers described previously (Bilic et al., 2009) in a reverse transcriptase polymerase chain reaction (RT-PCR). Briefly, external primers set Forw1_C-BLSV (5'-GGTATGGTTGATTTTGCCATAAAG-3') and Rev1_C-BLSV (5'-GCTGCNCGNARCAGTGTCGA-3') were used. The reverse transcriptase reaction and polymerase chain reaction were performed with the OneStep RT-PCR kit (Promega, France), according to manufacturer's instructions under the following conditions: 50°C for 30 min; 95°C for 5 min; 45 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, followed by a final elongation step of 72°C for 10 min. The negative control was water treated in the same way as the liver samples. Polymerase chain reaction (PCR) products with the expected size (280 bp) were revealed on a 1% agarose gel containing SybrGreen (Figure 2).

Statistical analysis

We performed the statistical analysis using R software version 2.13.0, through the package 'Rcmdr' version 2.5-1 (Fox et al., 2018). The differences in avian HEV RNA positivity between different variables (Locality and Species) were evaluated using

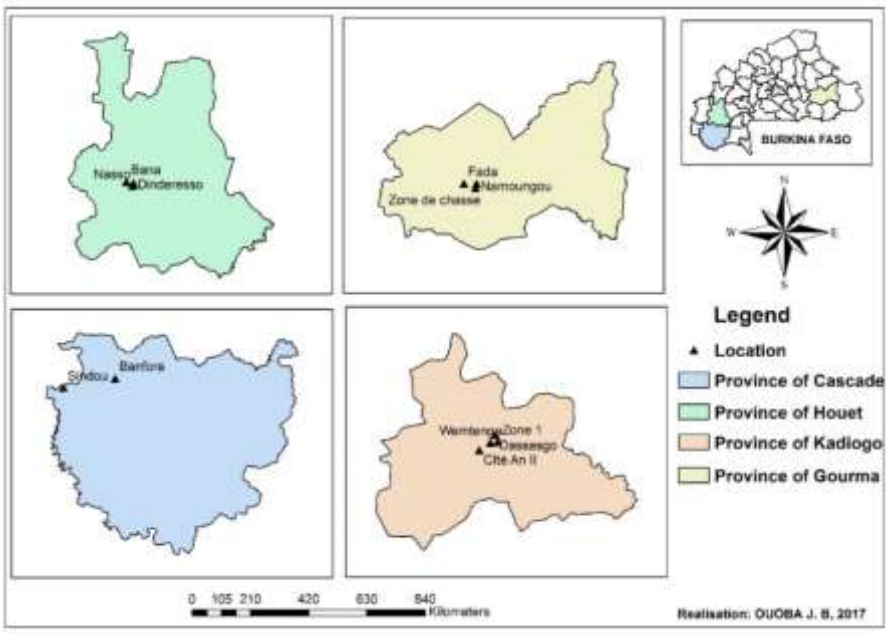

Figure 1. Geographical distribution of regions of sampling collection.

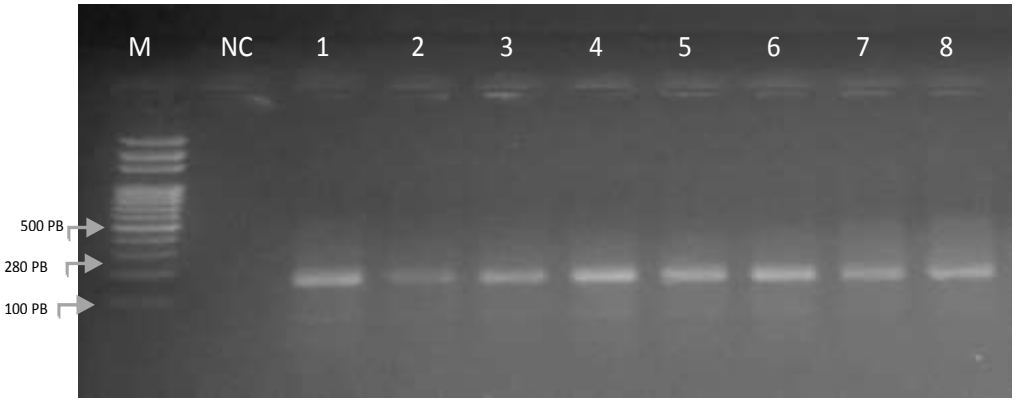

Figure 2. Electrophoresis result of aHAV.
Lane M: 100 bp DNA marker; NC: Negative controle; lane 1 to 8: aHEV positive samples.

logistic regression binomial. The best model was judged by Fisher's scoring algorithm. All tests were two-sided, and values of $p < 0.05$ were considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were estimated to explore the strength of the association between aHEV positivity and the conditions investigated.

RESULTS

Avian HEV RNA were detected in 29 (16.8 and 95% CI [11.2 – 22.3]; $p=2.2.10^{-16}$) of the 173 examined bird liver samples by RT-PCR (Tables 1 and 2). Of these, the prevalence was diverse in different birds species; the most frequent level was 35.3% (12/34, 95% CI [19.2 – 51.4]) in *N. meleagris*, followed by 23.5% (8/34, 95% CI [9.3 – 37.8]), in *G. gallus domesticus*; 13.3% (4/30, 95% CI [1.2 – 25.5]), in *S. turtur*; 13.3% (2/15, 95% CI [0 – 30.5]), in *C. livia*; 6.7% (2/30, 95% CI [0 – 15.6]), in *A. platyrhynchos* and 3.3% (1/30, 95% CI [0 – 9.8]) in *P. natalensis*. The highest proportions of positive samples were found in the domestic species 21.2% (24/113 95% CI [13.7 – 28.8], $p=9.7.10^{-10}$) against 8.3% in wild birds (5/60, 95% CI, [1.3 – 15.3], $p=1.1.10^{-10}$): Domestic birds had 4.8-fold higher risk than wild birds ([OR], 4.8; 95% CI, [1.8 – 15.9]; $p=4.0.\ 10^{-3}$) (Table 2).

Comparison between domestic and wild birds within the area where both species were tested in sufficient numbers, show that the prevalence of *N. meleagris* (3/3

Table 1. Detection of avian HEV RNA in domestic birds from Burkina Faso.

Species	Total number tested	Positive for aHEV (%)	p- value
Gallus gallus domesticus	34	8 (23.5)	1[a]
Numida meleagris	34	12 (35.3)	X, Y, Z* [a]
Anas platyrhynchos	30	2 (6.7)	1[a]
Columba livia	15	2 (13.3)	0,2571[a]
Total domestic bird	113	24 (21.2%)	0.030 [b]*

Table 2. Detection of avian HEV RNA in wild birds from Burkina Faso.

Species	Total number tested	Positive for aHEV (%)	p- value
Pternistis natalensis	30	1 (3.3)	1[a]
Streptopelia turtur	30	4 (13.3)	-
Total wild bird	60	5 (8.3)	

Note: (a): value of Fisher's exact test of independence. *X: comoé-gourma* p=0.2. *Y: comoé*-Kadiogo p=0.6. Z*: *Gourma-Kadiogo* $p=3.3.10^{-2}$. (b): value of Chi-Square test for Independence. Statistically significant p-values are less than 0.05. * Represent statistically significant value of χ^2.

(100%)) is higher than that of *S. turtur* (4/30 (13.3%)) in Gourma ($p< 0.01$).

In addition, within a specie, the positive rates of avian HEV RNA in liver varied according different locations; thus, *N. meleagris* in the district of Gourma are more likely to be infected than those in province of Kadiogo ($p< 0.05$). The total positive cases in a locality, without distinction of species, were respectively 18.2% (18/95 95% CI [10.6 – 25.8]) in the district of Kadiogo ($p=1.42.10^{-9}$), 23.3% (7/30 95% CI [8.2 – 38.5]; $p=3.5.10^{-3}$) in the district of Gourma, 6.2% (2/32 95% CI [0 – 14.6]; $p=6.0.10^{-4}$) in the district of Houet, and 16.7% (2/12 95% CI [0 – 37.5]; $p=2.1.\ 10^{-4}$) in the district of Comoé.

Thus, without distinction of species, in district of Kadiogo seems to have an approximately 1.85-fold higher risk than Comoé (odds ratio [OR], 1.8; 95% confidence interval [CI], [0.4 – 12.9]; p=0.4). Kadiogo had an approximately 0.7-fold lower risk than Gourma (OR, 0.7; 95% CI, [0.2 – 2.0]; p=0.5). Kadiogo had an approximately 5.4-fold higher risk than Houet (OR, 5.4; 95% CI, [1.4 – 35.8]; $p=3.2.10^{-2}$).

DISCUSSION

Evidence of aHEV infection of poultry has been well documented from the United States, Canada, China, Australia, Israel, and several countries in Europe (Haqshenas et al., 2001; Swayne, 2003; Agunos et al., 2006; Guo et al., 2006; Peralta et al., 2009; Xiao et al., 2013; Zhang et al., 2017). This study represents the first report on the distribution and molecular characterization of avian Hepatitis E Virus in domestic and wild bird without clinical symptoms in Burkina Faso. The overall aHEV RNA prevalence was 16.8% (29/173) in six birds species sampled from four districts of Burkina Faso, which was lower than that in chickens in the United States (29.9%; Gerber et al., 2014), Brazil (20.0%; Billam et al., 2005), and Korea (28%; Kwon et al., 2012), by ELISA, in China (30.6%) by Reverse transcription-polymerase chain reaction (RT-PCR), and (35.1%; Sun et al., 2016) by ELISA. The low prevalence recorded in detecting avian HEV genome could be attributed to sampling of apparently healthy birds and alternatively to the primers used (Gerber et al., 2015), as the avian HEV genome shows a high variability (Sprygin et al., 2012). Besides, detection rate of aHEV RNA in the pooled fecal samples was 62.9% (39/62) (Gerber et al., 2015), hence fecal samples could be another samples source, suitable for the molecular detection of avian HEV.

This prevalence of aHEV RNA was diverse in different birds species; the most frequent level was 35.3% in *N. meleagris*, 23.5%, in *G. gallus domesticus*, 13.3%, in *S. turtur*, 13.3%, in *C. livia*, 6.7% in *A. platyrhynchos* and 3.3% in *P. natalensis*. The differences could be related to differences in ecological and geographical factors (Cong et al., 2014). Thus, the high rate of aHEV RNA showed in the domestic species (*G. gallus domesticus, N. meleagris, A. platyrhynchos and C. livia*), could be due the poultry were highly congested in livestock areas, feces likely serve as the main source for virus spread within the flock (Haqshenas et al., 2001; Saif et al., 2008; Ahmad et al., 2010; Meng, 2011; Yugo et al., 2016). Domestic birds could be most often subject to a re-infection, because proper sanitation conditions in the henhouse are lacking and bird drinking water can contain feces (Crespo et al., 2015). This results suggests the possibility of aHEV transmission from asymptomatic

cases or repeated introduction through an unknown common source (Hsu and Tsai, 2014). Some studies have also shown that the high density of poultry increases the risk of disease transmission (Ricard and Marche, 1988). The low prevalence of aHEV RNA observed in wild birds (*P. natalensis* and *S. turtur*) also reflects that the congesting increases the likelihood of positive. Indeed, these birds live in liberty and are less congested compared to domestic birds. As for, *A. platyrhynchos* living in semi-liberty, the rate of positive sample (6.7%) was higher than in wild birds (Crespo et al., 2015). Burkina Faso is a developing country with low health and educational standards. Utilization of untreated bird feces for agriculture could increase the risk of virus dissemination, which in turn can infect wild birds. The high frequency of aHEV occurrence in bird livers in our country must be monitored to avoid an eventual outbreak. We have not investigated the source of aHEV infection in this study, but the role of wildlife in spreading the disease cannot be ignored (Crespo et al., 2015). The present study demonstrates the circulation of avian HEV in the domestic and wild birds without clinical symptoms, in Burkina Faso. This asymptomatic circulation of the virus in birds is of great interest and should be better monitored to avoid large epidemics. Thus we have to undertake studies on public health issues related to aHEV and the genetic diversity of aHEV inside the country.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agunos AC, Yoo D, Youssef SA, Ran D, Binnington B, Hunter DB (2006). Avian hepatitis E virus in an outbreak of hepatitis-splenomegaly syndrome and fatty liver haemorrhage syndrome in two flaxseed-fed layer flocks in Ontario. Avian Pathology 35(5):404-412.

Ahmad T, Waheed Y, Tahir S, Safi SZ, Fatima K, Afzal MS, Qadri I (2010). Frequency of HEV contamination in sewerage waters in Pakistan. The Journal of Infection in Developing Countries 4(12):842-845.

Bilic I, Jaskulska B, Basic A, Morrow CJ, Hess M (2009). Sequence analysis and comparison of avian hepatitis E viruses from Australia and Europe indicate the existence of different genotypes. Journal of General Virology 90(4):863-873.

Billam P, Huang FF, Sun ZF, Pierson FW, Duncan RB, Elvinger F, Meng XJ (2005). Systematic pathogenesis and replication of avian hepatitis E virus in specific-pathogen-free adult chickens. Journal of Virology 79(6):3429-3437.

Cong W, Meng QF, Shan XF, Sun WW, Qin SY, Zhang XX, Qian AD (2014). Seroprevalence and risk factors associated with hepatitis E virus infection in three species of pet birds in northwest China. Scientific World Journal 2014:296285.

Crespo R, Opriessnig T, Uzal F, Gerber PF (2015). Avian Hepatitis E Virus Infection in Organic Layers. Avian Disease 59(3):388-393.

Fox J, Bouchet-Valat M, Andronic L, Ash M, Boye T, Calza S, Chang A, Grosjean P, Heiberger R, Pour KK (2018). "Package 'Rcmdr'." http://cran.ma.imperial.ac.uk/web/packages/Rcmdr/Rcmdr.pdf

Gerber PF, Trampel DW, Opriessnig T (2014). Identification and characterization of avian hepatitis E virus in 2013 outbreaks of hepatitis-splenomegaly syndrome in two US layer operations. Avian Pathology 43(4):357-363.

Gerber PF, Trampel DW, Willinghan EM, Billam P, Meng XJ, Opriessnig T (2015). Subclinical avian hepatitis E virus infection in layer flocks in the United States. The Veterinary Journal 206(3):304-311.

Guo H, Zhou EM, Sun ZF, Meng XJ, Halbur PG (2006). Identification of B-cell epitopes in the capsid protein of avian hepatitis E virus (avian HEV) that are common to human and swine HEVs or unique to avian HEV. Journal of General Virology 87(1):217-223.

Haqshenas G, Shivaprasad HL, Woolcock PR, Read DH, Meng XJ (2001). Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. Journal of General Virology 82(10):2449-2462.

Hsu IW, Tsai HJ (2014). Avian hepatitis E virus in chickens, Taiwan, 2013. Emerging Infectious Diseases 20(1):149-151.

Kwon HM, Sung HW, Meng XJ (2012). Serological prevalence, genetic identification, and characterization of the first strains of avian hepatitis E virus from chickens in Korea. Virus Genes 45(2):237-245.

Meng XJ (2010). Hepatitis E virus: animal reservoirs and zoonotic risk. Veterinary Microbiology 140(3-4):256-265.

Meng XJ (2011). From barnyard to food table: the omnipresence of hepatitis E virus and risk for zoonotic infection and food safety. Virus Research 161(1):23-30.

Moon HW, Lee BW, Sung HW, Yoon BI, Kwon HM (2016). Identification and characterization of avian hepatitis E virus genotype 2 from chickens with hepatitis-splenomegaly syndrome in Korea. Virus Genes 52(5):738-742.

Park SJ, Lee BW, Moon HW, Sung HW, Yoon BI, Meng XJ, Kwon HM (2015). Construction of an infectious cDNA clone of genotype 1 avian hepatitis E virus: characterization of its pathogenicity in broiler breeders and demonstration of its utility in studying the role of the hypervariable region in virus replication. Journal of General Virology 96(5):1015-1026.

Pavio N, Meng XJ, Renou C (2010). Zoonotic hepatitis E: animal reservoirs and emerging risks. Veterinary Research 41(6):46.

Payne CJ, Ellis TM, Plant SL, Gregory AR, Wilcox GE (1999). Sequence data suggests big liver and spleen disease virus (BLSV) is genetically related to hepatitis E virus. Veterinary Microbiology 68(1-2):119-125.

Peralta B, Biarnes M, Ordonez G, Porta R, Martin M, Mateu E, Meng XJ (2009). Evidence of widespread infection of avian hepatitis E virus (avian HEV) in chickens from Spain. Veterinary Microbiology 137(1-2):31-36.

Purdy MA, Sue A (2017). The effect of phylogenetic signal reduction on genotyping of hepatitis E viruses of the species Orthohepevirus A. Archives of Virology 162(3):645-656.

Ricard F, Marche G (1988). Influence de la densité d'élevage sur la croissance et les caractéristiques de carcasse de poulets élevés au sol. Annales de zootechnie.

Ritchie SJ, Riddell C (1991). British Columbia. "Hepatitis-splenomegaly" syndrome in commercial egg laying hens. The Canadian Veterinary Journal 32(8):500-501.

Saif YM, Glisson JR, McDougald LR, Nolan LK, Swayne DE (2008). Diseases of Poultry. Blackwell Publishing Ltd, 12th Edition pp. 443-452.

Smith DB, Simmonds P, Study G, Jameel S, Emerson SU, Harrison TJ, Purdy MA (2015). Hepeviridae Consensus proposals for classification of the family Hepeviridae. Journal of General Virology 96(5):1191-1192.

Spahr C, Knauf-Witzens T, Vahlenkamp T, Ulrich RG, Johne R (2018). Hepatitis E virus and related viruses in wild, domestic and zoo animals: A review. Zoonoses Public Health 65(1):11-29.

Sprygin AV, Nikonova ZB, Zinyakov NG (2012). Avian hepatitis E virus identified in Russian chicken flocks exhibits high genetic divergence based on the ORF2 capsid gene. Avian Pathology 41(5):459-463.
Sridhar S, Teng JL, Chiu TH, Lau SK, Woo PC (2017). Hepatitis E virus genotypes and evolution: emergence of camel hepatitis E variants. International Journal of Molecular Sciences 18(4):869.
Sun Y, Du T, Liu B, Syed SF, Chen Y, Li H, Zhao Q (2016). Seroprevalence of avian hepatitis E virus and avian leucosis virus subgroup J in chicken flocks with hepatitis syndrome, China. BMC Veterinary Research 12(1):261.
Sun ZF, Larsen CT, Dunlop A, Huang FF, Pierson FW, Toth TE, Meng XJ (2004). Genetic identification of avian hepatitis E virus (HEV) from healthy chicken flocks and characterization of the capsid gene of 14 avian HEV isolates from chickens with hepatitis-splenomegaly syndrome in different geographical regions of the United States. Journal of General Virology 85(3):693-700.
Swayne DE (2003). Vaccines for list a poultry diseases: emphasis on avian influenza. Developments in Biologicals 114: 201-212.
Thiry D, Mauroy A, Pavio N, Purdy MA, Rose N, Thiry E, de Oliveira-Filho EF (2017). Hepatitis E Virus and Related Viruses in Animals. Transboundary and Emerging Diseases 64(1):37-52.
Wang X, Zhao Q, Dang L, Sun Y, Gao J, Liu B, Zhou EM (2015). Characterization of Two Novel Linear B-Cell Epitopes in the Capsid Protein of Avian Hepatitis E Virus (HEV) That Are Common to Avian, Swine, and Human HEVs. Journal Virology 89(10):5491-5501.
Ward JWA, Koh HK (2011). World Hepatitis Day: a new era for hepatitis control. The Lancet 378(9791):552-553.
Xiao CT, Gimenez-Lirola LG, Gerber PF, Jiang YH, Halbur PG, Opriessnig T (2013). Identification and characterization of novel porcine astroviruses (PAstVs) with high prevalence and frequent co-infection of individual pigs with multiple PAstV types. Journal of General Virology 94(3):570-582.
Yugo DM, Hauck R, Shivaprasad HL, Meng XJ (2016). Hepatitis Virus Infections in Poultry. Avian Diseases 60(3):576-588.
Yugo DM, Meng XJ (2013). Hepatitis E virus: foodborne, waterborne and zoonotic transmission. International Journal Environment Research Public Health 10(10):4507-4533.
Zhang X, Bilic I, Marek A, Glosmann M, Hess M (2016). C-Terminal Amino Acids 471-507 of Avian Hepatitis E Virus Capsid Protein Are Crucial for Binding to Avian and Human Cells. PLoS One 11(4):e0153723.
Zhang X, Bilic I, Troxler S, Hess M (2017). Evidence of genotypes 1 and 3 of avian hepatitis E virus in wild birds. Virus Research 228:75-78.
Zuin M, Caserta C, Romano L, Mele A, Zanetti A, Cannatelli R, Battezzati PM (2017). Seroepidemiology of HEV and HAV in two populations with different socio-economic levels and hygienic/sanitary conditions. European Journal of Clinical Microbiology and Infectious Diseases 36(3):479-485.

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Comparative cost analysis of three injectable ivermectin preparations in the control of gastrointestinal nematodes of sheep in Makurdi, Benue State Nigeria

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The efficacy and comparative cost analysis of three injectable ivermectin preparations was evaluated in West African Dwarf (WAD) sheep naturally infected with gastrointestinal nematodes. Three anthelmintics: ivomec classic® (IVC), ivomec super® (IVS) and ivomec gold® (IVG) were administered at a dose rate of 200µg/kg to control gastrointestinal nematodes in three treatment groups comprising five animals each. The faecal egg counts (FEC) for each animal pre-treatment, and thereafter for a period of 16 weeks post-treatment was carried out using the modified McMaster technique. The results is a pre-treatment mean FEC for groups A, B and C of 970±550.36, 880± 279.55 and 1640±893.78 eggs per gram (epg), respectively and a mean FEC of zero for all treatment groups one week post treatment. The mean FEC of zero was maintained for 28, 35 and 56 days, respectively. A mean FEC threshold for re-treatment of 500 epg was exceeded at days 42, 49 and 84 for groups A (615±167.26), B (830±287.49) and C (737.5±448.10), respectively. The results were subjected into a deterministic model to estimate the costs of using IVC, IVS or IVG in an annual control program. The costs of a one-time treatment were $20.6, $20.8 and $21.0, respectively. The average annual costs were $82.39, $83.22 and $41.99 for groups A, B and C, respectively. Thus, veterinary service and labour are two variables that contributed more to cost of treatment when compared with the price of drugs and average weight of the animals treated.

Key words: Ivermectin, gastrointestinal nematodes, West African Dwarf sheep, efficacy, cost analysis.

INTRODUCTION

Gastrointestinal (GI) parasite infection is considered as the most important limiting factor to sheep productivity in

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most parts of the world especially in developing countries (Waller, 1997; Roeber et al., 2013; Blackie, 2014; Singh et al., 2017a, b). The most common GI parasitic diseases in sheep in Nigeria are Haemonchosis, Strongyloidosis, Oesophagostomosis, Bunostomosis and Trichostrongylosis.

Haemonchus contortus has been singled out as the most important nematode of small ruminants in the tropics (Adamu et al., 2013; Singh et al., 2013; Zvinorova et al., 2016). Gastrointestinal nematode infections poses serious economic consequences to small ruminant production due to the associated morbidity, mortality, veterinary service and cost of treatment, as well as costs of other control measures (Singla, 1995; Zinsstag et al., 1998; Nwosu et al., 2007).

In ruminant production systems, parasite control has consistently shown a very high correlation with increased production (Kumar et al., 2013; Kenyon et al., 2013). Gastrointestinal nematode control strategies are almost entirely on the use of anthelmintic. The frequent use and mismanagement of these drugs has led to development of wide-spread resistance to the major groups of anthelmintic except for monepantel (Pomroy, 2006; Molento, 2009; Adamu et al., 2013; Melaku et al., 2013).

Ivermectin (IVM) is a macrocyclic lactone with activity against GI and lung nematodes (Nolan, 2012; Campbell, 2012), as well as against ectoparasites of clinical relevance in domestic animals (Campbell et al., 1984; Shoop et al., 1995; Merola and Eubig, 2012). Ivermectin has extensive tissue distribution, low biotransformation and high plasma-GI recycling that guarantees its persistent activity. The broad spectrum of activity and wide margin of safety has made it a drug of choice for nematode and arthropod parasitism in cattle, sheep, goat, swine, dog and horses (Campbell et al., 1983). Consequently, IVM is the most widely used anthelmintic and this extensive use has led to the emergence of IVM-resistant nematode populations in several countries (Jackson and Coop, 2000; Waller, 2006; Pomroy, 2006; Molento, 2009). The efficacy of IVM against gastrointestinal parasites under different control strategies has been demonstrated (Kenyon et al., 2013).

Farmers and veterinarians should be interested in information concerning the cost analysis of using different drugs and strategies to help with decision making for better control options. The decision making process is dependent on the costs of the anthelmintic, veterinary service and labour. These three variables may change depending on cost of anthelmintic, efficacy and duration of action against GI nematodes.

Currently, there are three injectable IVM preparations in the market produced by Merial. These IVM preparations are ivomec classic®, ivomec super® and ivomec gold®. The preparations differ in composition, duration of action and price. The aim of this study was to determine the efficacy, duration of action and cost implications of these IVM preparations as demonstrated by faecal egg counts pre and post-treatment for a period of 16 weeks in a flock of sheep.

MATERIALS AND METHODS

Experimental animals and ivermectin preparations

A total of fifteen West African Dwarf sheep kept at the University Teaching and Research Farm, University of Agriculture, Makurdi were randomly selected for this experiment. The sheep included 7 rams and 8 ewes out of a herd of 35 sheep. The sheep were kept under the semi-intensive system of management. Pregnant ewes and lambs were excluded from this experiment.

The compositions of the three injectable IVM preparations used in this experiment were: Ivomec classic® (IVC) contains 1% m/v of ivermectin; Ivomec super® (IVS) contains 1% m/v of ivermectin and 10% clorsulon and Ivomec gold® (IVG) contains 3.15% m/v of ivermectin. All three formulations used were manufactured by Merial South Africa (Pty) Limited. The anthelmintic was administered at a dose rate of 200 µg of IVM per kilogram body weight according to manufacturer's instructions.

Experimental design

The fifteen sheep were randomly assigned to 3 treatment groups (A, B and C), with each group comprising 5 sheep. Sheep in group A were treated with IVC, while sheep in groups B and C were treated with IVS and IVG, respectively.

Prior to the administration of the IVM preparations, a baseline faecal examination was carried out to determine the faecal egg counts of individual sheep pre-treatment. The sheep were weighed individually using a Camry® weighing scale.

The months of August to October represent the second half of the rainy season including its peak. During this period, it is expected that *Haemonchus* L_3 are well established in grazing pasture to pose a sufficient challenge to the experimental animals. The IVM formulations were administered to the sheep in the different groups at the same dose rate of 200 µg/kg subcutaneously, as recommended by the manufacturer.

Sampling and determination of faecal egg counts

Following treatments, faecal samples were collected per rectum from all the sheep in each group once weekly for 16 weeks. Samples were placed in polythene bags, labeled and transported on ice packs to the laboratory for further processing and examination.

The faecal samples were examined for helminth eggs and the faecal egg counts (FEC) for each sample was determined using the Modified McMaster technique using saturated sodium chloride solution as the floatation medium (Hansen and Perry, 1990).

Data collection and analysis

Prior to treatment with the respective injectable IVM preparations, the FEC for each of the 5 sheep per group was examined and recorded. The mean and standard error of the mean (SEM) were calculated and recorded for each group. The FEC of each sheep in the three groups was determined once weekly for a duration of 16 weeks. The mean FEC for each group and the SEM was similarly calculated for each group weekly. The FEC of 500 epg was used as the cut-off value in this study for a repeat of treatment (the week in which the mean FEC exceeded 500 epg was referred to as "re-treatment week"). The time interval between the week of first treatment and the re-treatment week was regarded as the "duration

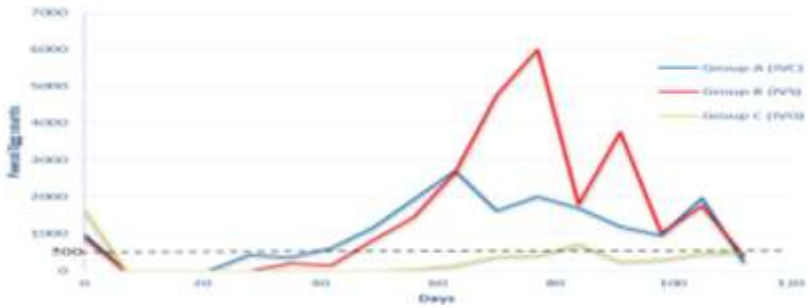

Figure 1. The mean faecal egg counts of sheep treated with three different ivermectin preparation for a 16 week period

of action" of the drug and it was noted for each group.

Economic analysis

A deterministic economic model was developed using Microsoft Excel to demonstrate the cost implications of using each of the IVM formulations with $_i$ representing the treatment groups (IVC, IVS and IVG). Table 2 shows the input values for variables used to develop the model. The calculated variables include: drug price per ml (dp_i), average weight of sheep in each group (AW_i), average dosage administered per group (DA_i), average cost of drug per treatment per group (Dc_i), veterinary service cost per group (VSc_i), labour cost per treatment per group (Lc_i), duration (weeks) till next treatment for each IVM preparation (DT_i) and number of treatments required per year (NT_i). The outcome of these calculations is the average annual cost of GI nematode control per group (ATC_i). VVc_i means veterinary visit cost.

Economic model calculations

Dc_i was calculated for each group by multiplying DA_i with dp_i as follows:

$$\mathrm{Dc}_i = \mathrm{DA}_i \times \mathrm{dp}_i \quad [1]$$

The average cost of a one-time treatment per group (Tc_i) is the sum of veterinary visit cost (VVc_i); and Dc_i, VSc_i and Lc_i for five sheep as follows:

$$\mathrm{Tc}_i = \mathrm{VVc}i + [\sum_{i=1}^{5} \mathrm{Dc}_i + \mathrm{VSc}_i + \mathrm{Lc}_i] \quad [2]$$

The NT_i was determined based on the fact that the rainy season provides an environment conducive for the proliferation of GI nematodes on pasture. The rainy season usually lasts about 6 months (24 weeks) during which several life-cycles of the parasites are expected to occur. Therefore, NT_i differed between groups based on their respective DA_i as follows:

$$\mathrm{NT}_i = \frac{24weeks}{DA_i} \quad [3]$$

The average annual cost of GI parasite control for one group (ATc_i) is the product of the Tc_i and the NT_i of the respective group.

$$\mathrm{ATc}_i = \mathrm{Tc}_i \times \mathrm{NT}_i \quad [4]$$

Sensitivity analysis

A sensitivity analysis was conducted in order to determine the level of impact of some variables on the annual cost of GI parasite control for each drug. This was done by adjusting the input values of certain variables by ±20% one at a time, while all other variables remained constant at their default values. The resulting average annual cost of GI parasite control (ATc_i) from each simulated scenario was compared with the default ATc_i calculated from the values from the field experiment. Variables for which sensitivity analysis was done include: veterinary visit cost (VVc_i), veterinary service cost (VSc_i), labour cost (Lc_i), the price of the drugs (DP_i) and the average weight of the animals (AW_i).

RESULTS

The mean faecal egg count for the three groups of 5 sheep treated with the three different ivermectin preparations are illustrated in Figure 1. The pre-treatment mean FEC across the groups is as follows: 970 epg for group A, 880 epg for group B and 1640 epg for group C (Table 1). One week after treatment, all the animals in the three groups presented 0 epg on examination of faecal samples. This is indicative of the efficacy of all three IVM preparations 7 days post treatment as 100%.

The three IVM formulations delayed re-infection for different durations. Sheep in group A treated with IVC maintained 0 epg status until day 28 post treatment, sheep in group B treated with IVS maintained 0 epg status until day 35 post treatment and sheep in group C treated with IVG maintained 0 epg status until day 56 post treatment. This implied that following clearance of infection, IVC, IVS and IVG prevented patent infection for a period of 28, 35 and 56 days, respectively.

Garg et al. (2007) reported a mean FEC of 500 epg in sheep requires anthelmintic treatment. The results in Table 1 therefore indicate the need for re-treatment on day 42 following treatment using IVC, day 49 following treatment with IVS and day 84 following treatment with IVG. The period of importance for the control of *H. contortus* is the rainy season which lasts about 6 months (180 days) on average in the study area. This implies the use of IVC and IVS will require 4 treatments per year,

Table 1. Mean faecal egg count ± standard error of the mean for groups of 5 sheep treated with IVC (Group A), IVS (Group B) and IVG (Group C).

Day	Mean FEC ± standard deviation		
	Group A	Group B	Group C
0	970±550.36	880±279.55	1640±893.78
7	0±0	0±0	0±0
14	0±0	0±0	0±0
21	0±0	0±0	0±0
28	*430±175.78	0±0	0±0
35	365±171.32	*200±187.75	0±0
42	**615±167.26	155±64.42	0±0
49	1170±436.92	**830±287.49	0 ±0
56	1945±924.37	880±279.55	*50±25.82
63	"2720±1533.59	880±279.55	150±85.63
70	1630±768.21	4765±2879.71	400±242.90
77	2020±1018.90	"6005±4205.36	425±335.91
84	1700±711.34	1800±725.95	**"737.5±448.10
91	1205±440.77	3760±2244.35	256.25±130.50
98	960±678.49	1030±615.14	312.5±124.33
105	1970±905.62	1755±874.66	475±121.79
112	230±93.01	410±137.30	512.5±250.58

*Reinfection

while farmers that use IVG will require only 2 treatments per year (Table 2). Hence, on the basis of efficacy and duration of action, IVG appears to be the more preferable choice among these injectable IVM formulations for the control of GI nematodes in a flock of sheep.

The outcome of the economic analysis shows that the average cost of a one-time treatment (Tc) for GI nematode infection using IVC, IVS and IVG for a group of 5 sheep are $20.6, $20.8 and $21.0, respectively. While the average annual costs of GI nematode control using IVC, IVS and IVG for a group of 5 sheep are $82.39, $83.22 and $41.99, respectively, as shown in Table 2.

A 20% increase or decrease in the average weight (AW) of the animals had the lowest impact on the average annual costs of GI nematode control (ATc) for all groups by increasing or decreasing the ATcby $0.004 for group A, $0.12 for B, and $0.1 for group C. The 20% increase or decrease in veterinary service cost (VSc) had the highest impact on the ATc for all the groups by increasing or decreasing the ATc by $4 for group A, $4 for group B and $2 for group C. Similarly, a 20% increase or decrease in veterinary visit costs (VVc); labour cost (Lc) and drug price (dp) increased or decreased the ATc as follows: $3.2 for group A, $3.2 for group B and $1.6 for group C; $2 for group A, $2 for group B and $1 for group C; $0.04 for group A, $0.12 for group B and $0.1 for group C respectively (Figures 2, 3 and 4).

DISCUSSION

This study evaluated the efficacy of three different IVM preparations in the treatment of GI nematodes in sheep as well as the onset of parasite re-infection after treatment. The efficacy of all three IVM preparations 7 days post treatment was 100% further strengthens claims about the susceptibility of GI nematodes to IVM in this region of Africa by Idika et al. (2012). Similarly, Peña-Espinoza et al. (2014) reported a 100% efficacy of IVM against *H. contortus* in small ruminants in Denmark. This finding is contrary to the report of anthelmintic resistance (AR) to all known anthelmintic groups including IVM in South Africa by Van Wyk et al. (1999). The difference may be due to a large scale sheep farming in South Africa and other southern hemisphere countries like Australia and New Zealand (Pomroy, 2006; Leathwick and Besier, 2014). These large-scale farms use IVM more frequently in GI nematode control as compared to the predominant small holder sheep farming structure in Nigeria. Periodic evaluation of the efficacy of common anthelmintic and possible resistance development by GI parasites is nevertheless important since AR has developed in sheep to all known anthelmintics except for monepantel (Kaminsky et al., 2011).

The different formulations of IVM (IVC, IVS and IVG) following clearance of infection prevented re-infection for a period of 28, 35 and 56 days, respectively. These differences in duration for re-infection to occur between treatment groups can be attributable to the different concentrations of IVM in the preparations used. The concentration of IVM in IVG may be responsible for the prolonged anthelmintic effect of IVG resulting from an extended half-life of the drug in plasma of treated sheep

Table 2. Default input values of costs and prices used in the economic analysis of the costs of GI nematode control using three different IVM preparations in WAD sheep.

Parameter	Abbreviation	Default Value ($)			Source
		IVC	IVS	IVG	
Drug Price/500 ml	DP	29.18	91.23	148.28	^OVAH S/Africa
Drug Price/ml	Dp	0.06	0.18	0.3	Calculated
Dosage (ml/kg)	D	0.02	0.02	0.02	*Merial®
Average weight (kg)	AW	16.73	16.73	16.73	Calculated
Dose Administered (ml)	DA	0.3	0.3	0.3	Calculated
Drug cost/treatment/sheep	DCT	0.02	0.06	0.1	Calculated
Drug cost/treatment/5 sheep	Dc	0.1	0.3	0.5	Calculated
Vet visit cost	VVc	8	8	8	Authors
Veterinary service cost/sheep	VSc	2	2	2	Authors
Veterinary service cost/5 sheep		10	10	10	Calculated
Labour cost/5 sheep	Lc	2.5	2.5	2.5	Authors
Cost of one-time treatment	**Tc**	**20.6**	**20.8**	**21.0**	Calculated
Duration till re-Rx of flock (wk)	DT	6	7	12	As implied from Garg et al. (2007)
No. of Rx req./year	NT	4	4	2	Calculated
Average annual treatment costs	ATc	**82.39**	**83.22**	**41.99**	Calculated

^OVAH S/Africa: Onderstepoort Veterinary Academic Hospital, Pretoria South Africa. *Merial®: The direction on the leaflets for each of the drugs was followed. $R_{\text{-treatment, wk;week, yr:year;}}$. IVC Ivomec classic, IVS Ivomec super, IVG Ivomec gold

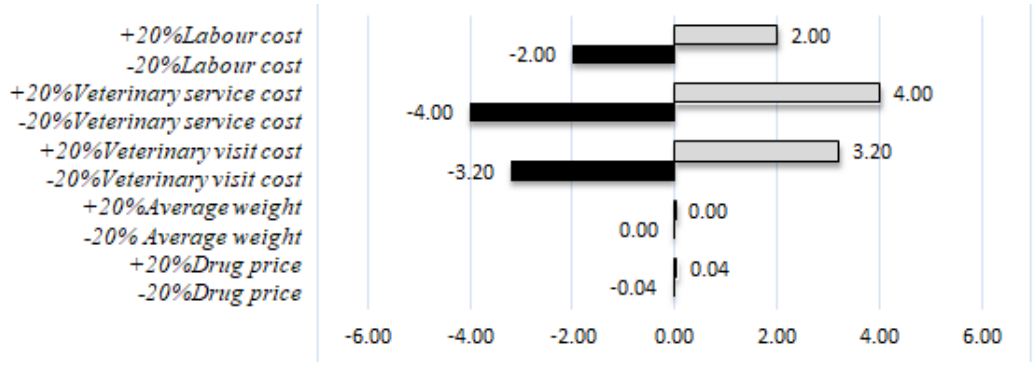

Figure 2. Turnado graph showing sensitivity analysis for Group A treated with Ivomec Classic®. Tornado graphs showing sensitivity analysis of cost variables. The values displayed are the differences between the average annual costs of GI nematode control in the normal scenario (with default prices for all cost variables) and the average annual costs of GI nematode control in a group of 5 WAD sheep under scenarios in which the value of one cost variable is altered. The negative values represent reduced marginal costs compared to the normal scenario, while the positive values represent additional marginal costs compared to the normal scenario in US Dollars.

(McKellar and Marriner, 1987; Garg et al., 2007).

The economic model used to evaluate the comparative cost analysis of the three IVM preparations shows that in spite of the relatively large difference in price between the drugs (that is, $119.10 between the cheapest option, IVC and the most expensive option, IVG), the consequential difference in the average costs of a one-time treatment for a group of 5 sheep was relatively small, that is, $1.40 between the cheapest option (IVC) and the most expensive option (IVG). This may be due to the proportion of treatment costs attributable to the cost of the drug used was only about 0.5, 1.5 and 2.4% for IVC, IVS and IVG, respectively. Whereas, other complementary costs that make up the treatment costs contributed much more. The proportion of treatment costs attributable to veterinary service cost is almost 50% for all three treatment options. A scenario manipulation of the model showed that the cost of veterinary services would account for as much as 75% of treatment costs in a flock of 50 sheep kept under the same circumstances as those in the current study. The relatively high cost associated with veterinary services make farmers to by-pass professionals there

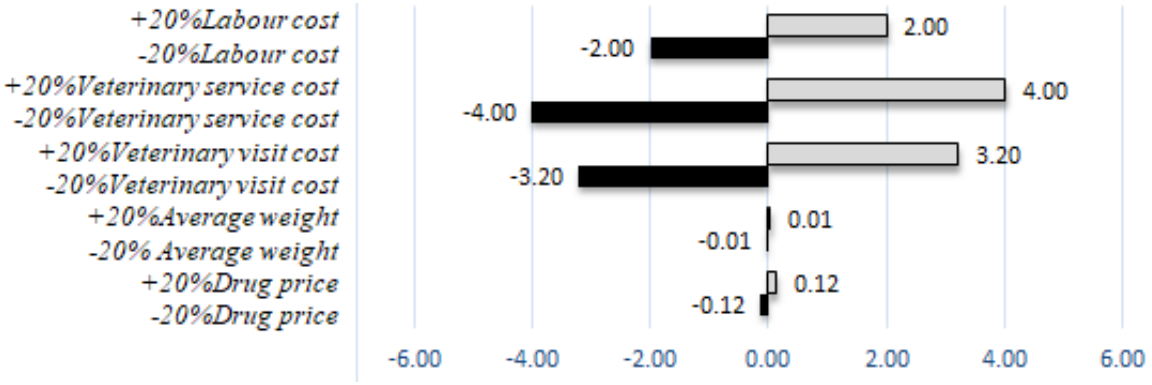

Figure 3. Turnado graph showing sensitivity analysis for Group B treated with Ivomec Super®. Tornado graphs showing sensitivity analysis of cost variables. The values displayed are the differences between the average annual costs of GI nematode control in the normal scenario (with default prices for all cost variables) and the average annual costs of GI nematode control in a group of 5 WAD sheep under scenarios in which the value of one cost variable is altered. The negative values represent reduced marginal costs compared to the normal scenario, while the positive values represent additional marginal costs compared to the normal scenario in US Dollars.

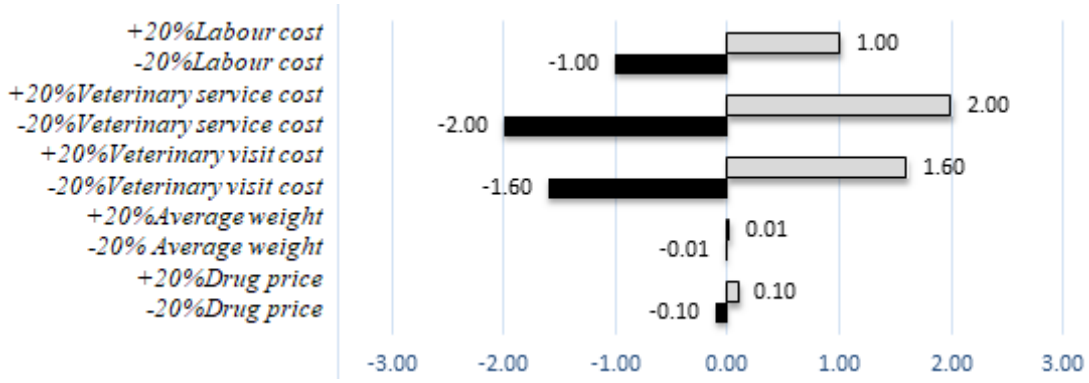

Figure 4. Turnado graph showing sensitivity analysis for Group C treated with Ivomec Gold®. Tornado graphs showing sensitivity analysis of cost variables. The values displayed are the differences between the average annual costs of GI nematode control in the normal scenario (with default prices for all cost variables) and the average annual costs of GI nematode control in a group of 5 WAD sheep under scenarios in which the value of one cost variable is altered. The negative values represent reduced marginal costs compared to the normal scenario, while the positive values represent additional marginal costs compared to the normal scenario in US Dollars.

by administering the drugs or employing the services of unqualified individuals (quacks). This abuse of the veterinary profession may increase the risk of anthelmintic resistance as a result of the use of incorrect dosages and routes of administration, as well as the practice of sub-optimal control strategies. Most farmers do not consider the cost of their labour (time and energy spent on catching and restraining the sheep during treatment) into account. This is taken for granted, but this study considered this an important input as persons may be employed to perform the duty. The farmer may perform the duty himself, the time spent should be valued based on the value of other profitable activities he/she could have been engaged in during the period. In the current study, labour costs accounted for about 12% of treatment costs, which is much more than the cost of the drugs, indicating that they should not be overlooked. The sensitivity analysis buttresses the fact that veterinary services and labour costs are the highest contributors to the overall costs and should be considered more importantly in the choice of control strategy, rather than the price of the drug which is usually the major consideration by farmers.

The major reason for which IVG emerged as a cheaper option, this is less than half the cost of either IVC or IVS is when used for an annual control programme. The

sheep were protected for 12 weeks, indicating that the treatment procedure will only need to be carried out twice a year as against four times when compared with the other two options used. This reduction in the frequency of treatment cuts down the cost of the procedure such as veterinary services and labour by 50%. This will reduce the exposure of the anthelmintic and possibly delaying the onset of anthelmintic resistance. Famers may be more willing to employ the services of a vet when the costs of veterinary services are lowered. To optimize the cost of anthelmintic control, the choice of anthelmintic should be based on the efficacy and duration of action of the drug.

Conclusion

The GI nematode parasites in circulation among West African Dwarf (WAD) sheep in the study area may not have developed resistance to ivermectin. The IVG confers a longer duration of protection when compared with IVC and IVS formulations. While IVG is costly than both IVC and IVS in a one-time treatment of GI nematodes in sheep, it is much cheaper to use in an annual control programme of GI nematodes of sheep in the study area. Veterinary service and labour costs contributed more to treatment costs than the price of the anthelmintic in GI nematode control. Thus, the decision for choice of anthelmintic for optimization of the cost analysis of GI nematode control should be based on the efficacy and duration of action of the drug rather than its price.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adamu M, Naidoo V, Eloff JN (2013). Efficacy and toxicity of thirteen plant leaf acetone extracts used in ethnoveterinary medicine in South Africa on egg hatching and larval development of Haemonchus contortus. BMC Veterinary Research 9(1):38. https://doi.org/10.1186/1746-6148-9-38.

Blackie S (2014). A Review of the epidemiology of gastrointestinal nematode infections in sheep and goats in Ghana. Journal of Agricultural Science 6(4):109-118. https://doi.org/10.5539/jas.v6n4p109.

Campbell WC (2012). History of avermectin and ivermectin, with notes on the history of other macrocycliclactone antiparasitic agents.CurrentPharmaceuticalBiotechnology13:853–865. https://doi.org/10.2174/138920112800399095

Campbell WC, Burg RW, Fisher MH, Dybas RA (1984). The discovery of ivermectin and other avermectins. Pesticide Synthesis through Rational Approaches pp. 5-20. https://doi.org/10.1021/bk-1984-0255.ch001.

Campbell WC, Fisher MH, Stapley EO, Albers-Schönberg G, Jacob TA (1983). Ivermectin: a potent new antiparasitic agent. Science 221(4613):823-828. https://doi.org/10.1126/science.6308762.

Garg R, Kumar RR, Yadav CL, Banerjee PS (2007). Duration of anthelmintic effect of three formulations of ivermectin (Oral, Injectable and Pour-on) against multiple anthelmintic-resistant Haemonchus contortus in sheep. VeterinaryResearchCommunications 31(6):749-755. https://doi.org/10.1007/s11259-007-0054-z.

Hansen J, Perry B (1990). The epidemiology, diagnosis and control of gastro-intestinal parasites of ruminants in Africa.International Laboratory for Research on Animal Diseases Nairobi.

Idika IK, Okonkwo EA, Onah DN, Ezeh IO, N Iheagwam C, Nwosu CO (2012). Efficacy of levamisole and ivermectin in the control of bovine parasitic gastroenteritis in the sub-humid savanna zone of southeastern Nigeria. Parasitology Research 111(4):1683-1687. https://doi.org/10.1007/s00436-012-3007-6.

Jackson F, Coop RL (2000). The development of anthelmintic resistance in sheep nematodes. Parasitology 120(1984):S95–S107. https://doi.org/10.1017/S0031182099005740.

Kaminsky R, Bapst B, Stein PA, Strehlau GA, Allan BA, Hosking BC, Sager H (2011). Differences in efficacy of monepantel, derquantel and abamectin against multi-resistant nematodes of sheep. Parasitology Research 109(1):19-23. https://doi.org/10.1007/s00436-010-2216-0.

Kenyon F, McBean D, Greer AW, Burgess CGS, Morrison AA, Bartley DJ, Jackson F (2013). A comparative study of the effects of four treatment regimes on ivermectin efficacy, body weight and pasture contamination in lambs naturally infected with gastrointestinal nematodes in Scotland. InternationalJournal forParasitology:Drugs Drug Resistance 3:77-84. https://doi.org/10.1016/j.ijpddr.2013.02.001.

Kumar N, Rao TKS, Varghese A, Rathor VS (2013). Internal parasite management in grazing livestock. Journal of Parasitic Diseases https://doi.org/10.1007/s12639-012-0215-z.

Leathwick DM, Besier RB (2014). The management of anthelmintic resistance in grazing ruminants in Australasia - Strategies and experiences. Veterinary Parasitology 204(1):44-54. https://doi.org/10.1016/j.vetpar.2013.12.022.

McKellar Q, Marriner S (1987). Comparison of the anthelmintic efficacy of oxfendazole or ivermectin administered orally and ivermectin administered subcutaneously to sheep during the periparturient period. Veterinary Record 120(16):383-386. https://doi.org/10.1136/vr.120.16.383.

Melaku A, Bogale B, Chanie M, Fentahun T, Berhanu A (2013). Study on utilization and efficacy of commonly used anthelmintics against gastrointestinal nematodes in naturally infected sheep in North Gondar, North-Western Ethiopia. African Journal of Pharmacy and Pharmacology 7(12):679–684. https://doi.org/10.5897/AJPP2012.2942.

Merola VM, Eubig PA (2012). Toxicology of avermectins and milbemycins (macrocylic lactones) and the role of P-glycoprotein in dogs and cats. Veterinary Clinics of North America: Small Animal Practice https://doi.org/10.1016/j.cvsm.2011.12.005.

Molento MB (2009). Parasite control in the age of drug resistance and changing agricultural practices. Veterinary Parasitology 163(3):229-234. https://doi.org/10.1016/j.vetpar.2009.06.007.

Nolan T (2012). Macrocyclic lactones in the treatment and control of parasitism in small companion animals. Current Pharmaceutical Biotechnology 13:1078-1094. https://doi.org/10.2174/138920112800399167.

Nwosu CO, Madu PP, Richards WS (2007). Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. Veterinary Parasitology 144(1):118-124. https://doi.org/10.1016/j.vetpar.2006.09.004.

Peña-Espinoza M, Thamsborg SM, Demeler J, Enemark HL (2014). Field efficacy of four anthelmintics and confirmation of drug-resistant nematodes by controlled efficacy test and pyrosequencing on a sheep and goat farm in Denmark. Veterinary Parasitology 206(3-4):208-215. https://doi.org/10.1016/j.vetpar.2014.10.017.

Pomroy WE (2006). Anthelmintic resistance in New Zealand: a perspective on recent findings and options for the future.The New Zealand Veterinary Journal 54(6):265-270. https://doi.org/10.1080/00480169.2006.36709.

Roeber F, Jex AR, Gasser RB (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular

tools for exploring epidemiology and drug resistance - an Australian perspective. Parasites and Vectors 6:1. https://doi.org/10.1186/1756-3305-6-153.

Shoop WL, Mrozik H, Fisher MH (1995). Structure and activity of avermectins and milbemycins in animal health. Veterinary Parasitology 59(2):139-156. https://doi.org/10.1016/0304-4017(94)00743-V.

Singh R, Ba MS, Singla LD, Kaur P (2017a). Detection of anthelmintic resistance in sheep and goat against fenbendazole by faecal egg count reduction test. Journal of Parasitic Diseases 41 (2):463-466.

Singh E, Kaur P, Singla LD, Bal MS (2017). Prevalence of gastrointestinal parasitism in small ruminants in western zone of Punjab, India. Veterinary World 10(1):61-66.

Singh V, Varshney P, Dash SK, Lal HP (2013). Prevalence of gastrointestinal parasites in sheep and goats in and around Mathura, India. Veterinary World 6(5):260-262. https://doi.org/10.5455/vetworld.2013:260-262.

Singla LD (1995) A note on sub-clinical gastro-intestinal parasitism in sheep and goats in Ludhiana and Faridkot districts of Punjab. Indian Veterinary Medical Journal 19:61-62.

Van Wyk JA, Stenson MO, Van der Merwe JS, Vorster RJ, Viljoen PG (1999). Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. Onderstepoort Journal of Veterinary Research 66(4):273-284.

Waller PJ (1997). Sustainable helminth control of ruminants in developing countries. Veterinary Parasitology 71:195-207. https://doi.org/10.1016/S0304-4017(97)00032-0.

Waller PJ (2006). Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science and Technology https://doi.org/10.1016/j.anifeedsci.2005.08.007.

Zinsstag J, Ankers P, Ndao M, Bonfoh B, Pfister K (1998). Multiparasitism, production and economics in domestic animals in sub-Saharan West Africa. Parasitology Today 14(2):46-49. https://doi.org/10.1016/S0169-4758(97)01183-6.

Zvinorova PI, Halimani TE, Muchadeyi FC, Matika O, Riggio V, Dzama K (2016). Prevalence and risk factors of gastrointestinal parasitic infections in goats in low-input low-output farming systems in Zimbabwe. Small Ruminant Research 143:75-83. https://doi.org/10.1016/j.smallrumres.2016.09.005.

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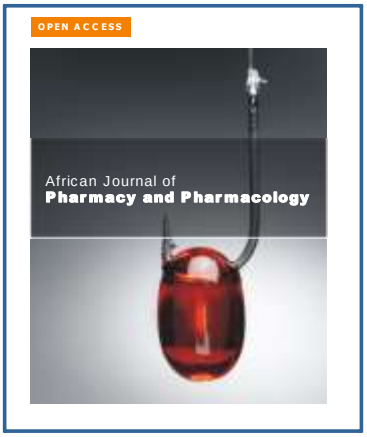

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